UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: February 23, 2015

SUBJECT: Trinexapac-ethyl: Human Health Risk Assessment to Support New Uses on Rice and Rye.

PC Code: 112602
Decision Nos.: 488373
Petition Nos.: NA
Risk Assessment Type: Single Chemical
TXR No.: NA
MRID No.: NA

DP Barcode: D419651
Registration Nos.: NA
Regulatory Action: Registration Review
Case No.: NA
CAS No.: 95266-40-3
40 CFR: §180.662

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The Health Effects Division (HED) of the Office of Pesticide Programs (OPP) is charged with estimating the risk to human health from exposure to pesticides. Registration Division (RD) of OPP has requested that HED evaluate the hazard and exposure data and conduct dietary,

occupational, residential, and aggregate exposure assessments, as needed, to estimate the risk to human health that will result from the registered uses of trinexapac-ethyl on rice and rye.

The HED team members contributing to this risk assessment include Monica Hawkins (occupational and residential assessment), Nancy McCarroll (hazard assessment), Sumitra Biswas (chemistry and dietary assessment), and Sheila Piper (risk assessment). Christopher Koper of the Environmental Fate and Effects Division (EFED) performed the drinking water assessment.

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1.0 Executive Summary

HED has conducted a human health risk assessment to support requested Section 3 uses for the active ingredient, trinexapac-ethyl, a plant growth regulator/herbicide that is registered for uses on cereal grains (wheat, barley, oats, triticale), sugarcane and grasses grown for seeds. The requested action is to establish permanent tolerances for residues of trinexapac-ethyl on rice and rye. It is formulated as an emulsifiable concentrate, wettable powder packed in water-soluble bags, and granule. Trinexapac-ethyl is used on turf in residential and commercial lawns, golf courses, parks, athletic fields, cemeteries, as well as grass grown for seed (non-food) and turf grown for sod.

The toxicological database for trinexapac-ethyl is complete. Trinexapac-ethyl exhibits low acute toxicity as shown in the standard acute toxicity battery as well as in the acute neurotoxicity study in rats with no systemic or neurotoxic effects up to the limit dose. The dog appears to be the most sensitive species while no systemic adverse effects were seen in rats, rabbits, or mice up to the limit dose (1,000 mg/kg/day) following subchronic or chronic oral exposure. Data from the combined chronic toxicity/carcinogenicity study in the rat did not demonstrate an increase in any tumor type that would be relevant to humans. The immunotoxicity study showed no evidence of immunotoxicity up to the limit dose. Trinexapac-ethyl is classified as "Not likely to be Carcinogenic to Humans." Therefore, aggregate cancer risk was not quantitatively assessed.

HED recommends that the 10x FQPA Safety Factor (for the protection of infants and children) be reduced to 1x. The toxicity database is complete and there is no evidence of neurotoxicity in the database. While there is evidence of increased qualitative and quantitative susceptibility in the rat (increased incidence of asymmetrical sternebrae at the limit dose) and rabbit (decreased number of live fetuses/litter and increased post-implantation loss and early resorptions) at doses in the absence of maternal toxicity, these effects occurred only at the highest doses tested, are well characterized, and there is a clear NOAEL for these effects. Therefore there is no residue concerns with respect to developmental and reproductive effects. Lastly, the exposure estimates used to assess risk are unlikely to underestimate exposure.

An endpoint for the incidental oral exposure scenario was not identified from the available trinexapac-ethyl toxicity studies. For short- and intermediate-term dermal and inhalation exposures, a rabbit developmental toxicity study was selected with a NOAEL of 60 mg/kg/day and a LOAEL of 360 mg/kg/day based on a decrease in mean number of fetuses/litter and an increase in post-implantation loss. This endpoint is only applicable to adults (females) because the endpoint is based on *in utero* effects. HED reviewed the full toxicological database for trinexapac-ethyl in an attempt to select endpoints for the dermal and inhalation exposure scenarios for children; however, no appropriate endpoint was identified. A target MOE of 100 is considered adequate for all exposure scenarios (10x for interspecies extrapolation, 10x for intraspecies variability).

The residue chemistry database for trinexapac ethyl is complete. The nature of the residue in plants and livestock is adequately understood, and the residue of concern in primary crops, rotational crops, and livestock for both tolerance enforcement and risk assessment include free and conjugated residues of both parent trinexapac-ethyl and its acid metabolite, trinexapac. In drinking water, the degradates of concern for risk assessment are trinexapac-ethyl, trinexapac, 2-(4-cyclopropyl-4-hydroxy-2-oxobutyl) succinic acid (CGA-313458), an open chain cyclohexane ring, and the unidentified hydroproduct M3. Rice field trials have been submitted which are adequate in number and geographic representation, generated by a validated analytical method, and supported by adequate storage stability data. An acceptable processing study has been provided which shows that residues of trinexapac-ethyl concentrate in polished rice and bran but do not concentrate in hulls. There are no livestock feed items associated with this petition; therefore a discussion of the magnitude of residues in livestock commodities, as well as the need for livestock tolerances and analytical methods is not pertinent to this discussion.

Highly conservative acute and chronic dietary (food and drinking water) risk assessments were conducted using tolerance-level residues, assuming 100% crop treated for all commodities, using default processing factors, and incorporating modelled drinking water estimated exposure values. The acute dietary food and drinking water risk estimates are 2% of the acute population-adjusted dose (aPAD) for females 13-49 years old and are below HED's level of concern (<100% of the acute population adjusted dose (aPAD)) at the 95th percentile of exposure. An acute dietary endpoint was not selected for the general population or any other population subgroups except females 13-49 years old. The chronic dietary risk estimate for food and drinking water is 3% of the chronic population-adjusted dose (cPAD) for the general U.S. population and 6% for children 1-2 years old, the population subgroup with the highest estimated chronic dietary exposure to trinexapac-ethyl.

No new residential uses are being proposed at this time so an updated residential exposure assessment is not required. There are registered residential uses for trinexapac-ethyl on turf that were assessed previously. The residential handler MOEs range from 240 to 21,000,000. The residential post-application MOEs range from 450 to 61,000.

The acute (females 13– 49 years only) and chronic aggregate assessments are equivalent to the corresponding dietary (food plus water) risk estimates, which do not exceed HED's level of concern (MOEs $\geq$ 100). The short/intermediate-term aggregate MOE for females 13– 49 years old is 230 and children (11 - 16 years old) is 4,500. There are no oral, dermal or inhalation hazards for children associated with trinexapac-ethyl; therefore an aggregate risk assessment is not required for this population subgroup.

In the previous occupational exposure assessment for trinexapac-ethyl, occupational handler and post-application exposures were assessed for the currently registered use for each trinexapac-ethyl crop, including cereal grains which was assessed at a maximum application rate of 0.11 lb ai/A. The maximum single application rates for both rice and rye are either the same or lower

than crops that were previously assessed. Therefore, an updated occupational exposure assessment for the proposed new uses is not required at this time. The occupational handler assessment MOEs range from 140 to 2,100,000 with baseline attire and/or with the addition of gloves (as required on the labels). The occupational post-application MOEs on the day of application range from 87 to 43,000. Based on HED's occupational post-application exposure assessment, the restricted-entry interval (REI) for hand-harvesting sugarcane was estimated to be 2 days for short- and intermediate-term exposure. However, HED does not recommend that sugarcane REI's be changed on the trinexapac-ethyl labels at this time based on two main issues. One, almost all sugarcane harvesting in the United States is done mechanically. Two, the proposed trinexapac-ethyl labels have a 28-day pre-harvest interval (PHI) for sugarcane, so hand harvesting should not occur until after 28 days which is protective of the estimated REIs (M. Hawkins, 9/18/13, D413030).

2.0 HED Recommendations

2.1 Data Deficiencies

There are no data deficiencies relating to toxicology and residue chemistry associated with the current petition for uses of trinexapac-ethyl. As previously stated in the last risk assessment (D413570), a DFR study will be required for future assessments for trinexapac-ethyl. In accordance with the updated Part 158 data requirements (2007), one or more DFR studies are required when a pesticide has residential or occupational uses that could result in post-application dermal exposure.

2.2 Tolerance Considerations

2.2.1 Enforcement Analytical Method

An adequate enforcement method is available for trinexapac-ethyl, Method GRM020.01A, which utilizes high performance liquid chromatography with triple-quadrupole mass spectrometry (LC-MS/MS) to support cereal grain uses. The method includes a strong acid hydrolysis/extraction procedure to release both "free" and "conjugated" residues of trinexapac acid from field grown grass commodities. The reported limit of quantitation (LOQ) is 0.010 ppm. Validated analytical methods are available for both data collection and enforcement purposes.

Also, trinexapac-ethyl was evaluated using the FDA multi-residue method Protocols C and D, and its acid metabolite, trinexapac, was evaluated using Protocol B. These data indicate FDA multi-residue methods are not suitable for determining residues of trinexapac-ethyl and trinexapac in plant commodities.

2.2.2 Recommended Tolerances

Permanent tolerances are currently established for residues of the plant growth regulator trinexapac-ethyl, including its metabolite and degradate, in or on the commodities in the 40 §CFR 180.662. The tolerance expression listed in the 40 CFR 180.662 complies with the HED interim tolerance expression policy (S. Knizner, 5/27/09). Permanent tolerances for trinexapac-ethyl should be established under 180.662(a) for the commodities listed below at the HED-recommended tolerance levels shown in Table 2.2.2.

Table 2.2.2. Tolerance Summary for Trinexapac-ethyl.

Commodity	Proposed Tolerance (ppm)	HED-Recommended Tolerance (ppm)	Comments (*correct commodity definition*)
Rice, grain	0.4	0.4	
Rice, straw	0.07	0.07	
Rice, bran	1.5	1.5	
Rice, wild, grain	0.4	0.4	
Rye, bran	2.5	6.0	
Rye, grain	2.0	4.0	
Rye, hay	0.8	1.5	
Rye, straw	0.4	0.9	

2.2.3 Revisions to Petitioned-For Tolerances

There are no changes to the petitioned-for tolerances for rice commodities. However, HED recommends revisions to the proposed rye tolerances. For rye, the registrant proposed tolerances by extrapolating residue data for barley. HED concurs with translating from the existing cereal grains, however, since tolerances for wheat commodities are higher than tolerances for barley commodities, and HED recommends setting tolerances for rye, based on the higher wheat tolerances.

2.2.4 International Harmonization

There are no established or proposed Codex, Canadian, or Mexican maximum residue limits (MRLs) for trinexapac-ethyl in or on any food or feed crops. However, a variety of European countries, as well as Argentina, Brazil, Japan, Korea, and Australia have established or proposed MRLs for trinexapac-ethyl on cereal grains (0.02-0.5 mg/kg), cereal grain forages and fodders (0.2-3 mg/kg), sugarcane (0.05-0.1 mg/kg), sugar forage and fodder (1 mg/kg), meat (0.02 mg/kg), and/or milk (0.1-0.005 mg/kg). At this time, there are no issues with respect to international harmonization associated with this petition.

2.3 Label Recommendations

Trinexapac-ethyl is classified as Toxicity Category III for acute oral, dermal, and eye irritation, and IV for acute inhalation and skin irritation. It is not a dermal sensitizer. Therefore, the acute toxicity categories for this chemical require a 12 hour restricted entry interval (REI) under 40 CFR 156.208 (c) (2) (iii).

3.0 Introduction

Trinexapac-ethyl is a cyclohexadione plant growth regulator currently registered for use on grasses grown for seed, cereal grains (barley, oats, triticale, and wheat) and sugarcane. Trinexapac-ethyl inhibits internodal elongation and growth in cereal grains, grasses and several dicotyledonous species. It is registered in numerous European countries and several Asian and Latin American countries for uses on cereal grains and sugarcane. Tolerances are currently established for residues of trinexapac-ethyl in or on food commodities under 40 CFR §180.662, at levels ranging from 0.02 to 40 ppm.

The most recent risk assessment was performed for registration review on September 12, 2013 (D413570; S. Piper, D. Smegal, M. Hawkins).

Syngenta Crop Protection has petitioned to establish a permanent tolerance for residues of trinexapac-ethyl in/on rice and rye. The registrant provided field trial data on the use of trinexapac-ethyl in/on rice commodities. The tolerances on rye was requested based on the November 25, 2009, ChemSAC decision that provides an allowance for extrapolating residue data from barley and wheat to additional small cereal grains. Since tolerance are established for cereals (wheat, barley, and oats), the tolerances for rye commodities can be translated from established tolerances on small cereal grains.

3.1 Chemical Identity

Table 3.1. Trinexapac-ethyl Compound Nomenclature

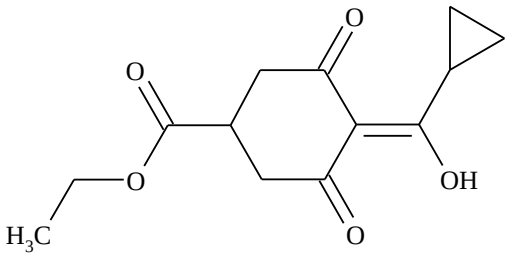
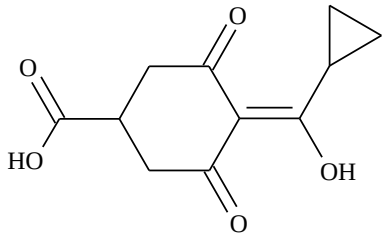
Compound	
Common name	Trinexapac-ethyl
Company experimental name	CGA163935
IUPAC name	ethyl (*RS*)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylate
CAS name	ethyl 4-(cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylate

Table 3.1. Trinexapac-ethyl Compound Nomenclature	
CAS registry number	95266-40-3
End-use product (EP)	1.0 lb/gal EC (Palisade™ EC; EPA Reg. No. 100-949) 2.0 lb/gal EC (Palisade 2EC; EPA Reg. No. 100-1241)
Regulated Metabolite	
Common Name	Trinexapac
Company Experimental Name	CGA-179500
IUPAC name	(*RS*)-4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylic acid
CAS name	4-(cyclopropylhydroxymethylene)-3,5-dioxocyclohexanecarboxylic acid
CAS registry number	104273-73-6

3.2 Physical/Chemical Characteristics

Trinexapac-ethyl has a relatively low octanol/water partition coefficient. At pH 5.3, the log K_{OW} value is 2.44 (K_{OW} = 275). Trinexapac-ethyl has a low potential to leach into groundwater and is not expected to be volatile because of its low vapor pressure (2.16×10^{-3} Pa at 25ºC). See Table 3.2 for a listing of trinexapac-ethyl physical and chemical properties.

Table 3.2. Physical and Chemical Properties of Trinexapac-Ethyl.		
Parameter	Value	Reference
Melting point/range	36.1-36.6°C	Provided in MRID 46809305
pH	3.3	
Density (20ºC)	1.215 g/cm^3	
Water solubility (g/L at 25°C)	2.8 at pH 4.9 10.2 at pH 5.5 21.1 at pH 8.2	
Solvent solubility	Acetone 100% Ethanol 100% Toluene 100% n-octanol 100% n-hexane 5%	
Vapor pressure (25ºC)	2.16x 10^{-3} Pa	
Dissociation constant, pK_a	4.57	
Octanol/water partition coefficient, Log(K_{OW}) at 25ºC	2.44 at pH 5.3	
UV/visible absorption spectrum	Neutral: 9335 L/mol·cm @ 240.2 nm 13976 L/mol·cm @ 277.4 nm Acidic: 11712 L/mol·cm @ 240.0 nm 12368 L/mol·cm @380.4 nm Basic: 21320 L/mol·cm @ 270.8 nm	

3.3 Pesticide Use Pattern

Trinexapac-ethyl is marketed as an emulsifiable concentrate, wettable powder packed in water-soluble bags, and granule. Syngenta Crop Protection has registered trinexapac-ethyl for multiple end-use products on turf areas such as sod farms, golf courses, sport fields, cemeteries, and similar areas, and on grasses grown for seed, sugarcane, and cereal grains (wheat, barley, oats, triticale). The label-required personal protective equipment (PPE) for occupational applicators and handlers is a long-sleeved shirt, long pants, shoes, and socks, chemical-resistant gloves, and protective eyewear. The currently proposed uses on rice and rye are requested only on the 1 lb ai/gal and 2 lb ai/gal EC formulations and are summarized in Table 3.3. HED notes that the requested use on rye is identical to the established uses for wheat and barley.

Table 3.3. Summary of Directions for the Proposed Uses of Trinexapac-ethyl							
Applic. Timing, Type, and Equip.	**EPA Reg. No. (formulation type)**	**Max. Single Application Rate (lb ai/A)**	**Max. No. Application per year**	**Max. Annual App. Rate (lb ai/A)**	**RTI (days)**	**PHI (days)**	**Use Directions and Limitations**
Rice							
Post-emergent aerial application to flooded rice fields	1.0 lb/gal EC 100-949 2.0 lb/gal EC 100-1241	0.045	1	0.045	NA	50	For best performance, apply a single application to one of the following growth stages: Fully tillered, panicle initiation, or panicle formation. Do not apply once internode is more than ½ to ¾ inch in length.
Rye (same use directions as other small grains/cereals currently on the labels)							
Post-emergent application (ground or aerial)	1.0 lb/gal EC 100-949 2.0 lb/gal EC 100-1241	0.11	1	0.11		45	Make applications from the Feekes growth stage 4 through Feekes growth stage 7. Split applications may be made at Feekes growth stage 4-5 and then again at Feeks 7 and 8. Apply in a min of 20 gal/A.
		0.05	2				

Conclusion
The proposed label for trinexapac-ethyl is adequate to allow evaluation of the residue data relatively to the proposed use on rice. Label direction for rye are equivalent to the currently registered uses of cereal grains for the same formulations.

3.4 Anticipated Exposure Pathways

Humans may be exposed to trinexapac-ethyl in food because trinexapac-ethyl may be applied directly to growing crops. In addition, these applications can result in trinexapac-ethyl reaching surface and groundwater, both of which can serve as sources of drinking water. Homeowners may be exposed to trinexapac-ethyl when applying to their lawns, and both adults

and children may be exposed when coming in contact with treated turf. In an occupational setting, applicators may be exposed while handling the pesticide prior to application, as well as during application. There is a potential for exposure for workers re-entering treated fields and coming in contact with treated foliage.

3.5 Consideration of Environmental Justice

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," (http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf. As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA's National Health and Nutrition Examination Survey, What We Eat in America, (NHANES/WWEIA) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age and ethnic group. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas postapplication are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

4.0 Hazard Characterization and Dose-Response Assessment

4.1 Toxicology Studies Available for Analysis

The toxicology database for trinexapac-ethyl is complete and adequate to support the proposed new uses. The Hazard and Science Policy Council (HASPOC) determined that the subchronic inhalation study is not required at this time (Dunbar, 2013, TXR # 0056699). Data requirements for the food uses of trinexapac-ethyl are listed in Table A.1 in Appendix A. The data from the following studies were used to evaluate the hazard potential of trinexapac-ethyl (see Appendix A: Tables A.2 and A.3):

- Acute lethality studies (oral, dermal, inhalation, primary eye and dermal irritation, and dermal sensitization)

- Acute and subchronic neurotoxicity studies (rat)
- Subchronic oral toxicity studies (rat and dog)
- Developmental (rat and rabbits) and reproductive toxicity (rat) studies
- Dermal toxicity (21-day dermal toxicity in rabbits)
- Chronic oral toxicity studies (rat and dog)
- Carcinogenicity study (mouse)
- Metabolism study (rat)
- Dermal penetration study (rat)
- Mutagenicity battery
- Immunotoxicity study (mouse)

4.2 Absorption, Distribution, Metabolism, & Elimination (ADME)

The metabolism study (MRID 41563927) was available and considered acceptable\guideline. In this study, groups of 5 male and 5 female rats received single oral doses of 0.97 or 166 mg/kg, or 0.91 mg/kg intravenously (i.v.) of trinexapac-ethyl. An additional group of males and females were pre-conditioned for 14 days with daily oral doses of ~1 mg/kg/day non-radiolabeled trinexapac-ethyl, followed by 7 daily doses of radiolabeled test material (~1 mg/kg/day).

4.2.1 Dermal Absorption

A single dermal dose of 0.01, 0.1, or 1.0 mg/cm^2 ^{14}C-trinexapac-ethyl administered to male rats was rapidly absorbed, distributed, and eliminated (MRID 42238105). The amount absorbed increased with duration of exposure. The average ^{14}C-trinexapac-ethyl absorbed within 24 hours was 56.8, 66.7, and 33.8% of the applied dose for the low-, mid-, and high- dose groups, respectively. Recovery of the applied dose was 97-117% and most was recovered from the skin washes and urine. The current policy is to use 8-hours exposure and to assume that residues found on the skin are potentially absorbable, unless data are provided to demonstrate otherwise. This policy reflects an 8-hour work day, and ongoing exposure is expected to cease at the end of the workday. Likewise, the Agency's position is to be conservative to ensure public safety, and this is reflected in the assumption that residues found on the skin will be absorbed, unless there are data which contradicts this assumption. Since absorption rates were not measured beyond 24 hours, the percent of the dose absorbed plus the percentage of the dose remaining on the washed application site (potentially absorbable) at 10 hours was 56.5% and 21%, respectively. Using the conservative approach, HED calculates the dermal absorption factor (DAF) to be 77.5% of the dose (0.01mg/cm^2) for trinexapac-ethyl.

4.3 Toxicological Effects

4.3.1 Toxicology Profile

Trinexapac-ethyl exhibits low acute toxicity as shown in the standard acute toxicity battery as well as in the acute neurotoxicity study in rats with no systemic or neurotoxic effects up to the limit dose. The dog appears to be the most sensitive species while no systemic adverse effects were seen in rats, rabbits, or mice up to the limit dose (1,000 mg/kg/day) following subchronic or chronic oral exposure. In the dogs; however, decreased body weight gain and food consumption, diffuse thymic atrophy, and changes in the epithelial cells of the renal tubules were seen in the 90-day dog study at 516/582 mg/kg/day (males/females). Following chronic exposure, dose-related neuropathology of the brain characterized as focal bilateral vacuolation of the dorsal medial hippocampus and/or lateral midbrain was seen at ≥365/357 mg/kg/day in male and female dogs, respectively. The lesions remained confined to the supporting cells in the central nervous system and did not progress to more advanced or more extensive damage of the nervous tissue. These lesions were not associated with other neuropathological findings or overt neurological signs, so their biological significance is unknown. Similar lesions were not observed in the rat or mouse following subchronic or chronic dietary exposure, and there was no other evidence in any other species tested to indicate a neurotoxicity potential. Furthermore, the brain lesions observed in the chronic dog study are not likely to develop from a short-term exposure and were not observed in either the rat or mouse short-term studies. In support of these findings, no evidence of neurotoxicity in the acute or subchronic rat neurotoxicity studies was found.

In the rat and rabbit developmental toxicity studies, there is evidence of increased qualitative and quantitative susceptibility in the rat (increased incidence of asymmetrical sternebrae at the limit dose) and rabbit (decreased number of live fetuses/litter and increased post-implantation loss and early resorption at 360 mg/kg/day) in the absence of maternal toxicity. Qualitative sensitivity was observed in the 2-generation reproduction study but only in excess of the limit dose (1,212 mg/kg/day). The decreased pup survival when analyzed with sexes combined, resulted in statistical significance (5-7%); this finding was not significant when the data were analyzed separately. Further evaluation of the individual litters suggested that one or two litters were the cause of the reduced pup survival at the highest dose tested. Reproductive toxicity was not observed up to the limit dose. There was also no indication of immunotoxicity in mice up to the limit dose.

Data from the combined chronic toxicity/carcinogenicity study in the rat did not demonstrate an increase in any tumor type that would be relevant to humans. The observation of squamous cell carcinomas in the non-glandular portion of the stomach of two males at 806 mg/kg/day does not provide reasonable evidence of a possible deleterious effect of trinexapac-ethyl on the pharynx and/or esophagus (non-glandular areas) of the human. This is because trinexapac-ethyl would not be in contact with human tissues for a significant period of time compared to the length of

time it was in contact with the non-glandular portion of the rat stomach. Follicular adenocarcinomas of the thyroid were significantly increased in males (5%) at 806 mg/kg/day but this value was within the historical control range. In the mouse, there was no evidence of carcinogenicity. The mutagenicity database is complete, with no evidence of mutagenicity. The cancer classification for trinexapac-ethyl is "Not Likely to be Carcinogenic to Humans."

4.4 Safety Factor for Infants and Children (FQPA Safety Factor)

The existing use pattern for trinexapac-ethyl could potentially result in dietary (various crops) and residential (turf) exposure to infants and children. Thus, FQPA hazard considerations were addressed in HED's evaluation of the toxicology database. HED recommends that the 10x FQPA Safety Factor (for the protection of infants and children) be reduced to 1x. The rationale for this decision is that the toxicological database for trinexapac-ethyl is complete with regard to pre-and postnatal toxicity, immunotoxicity, and neurotoxicity studies, and there are no residual uncertainties. Additionally, the dietary exposure assessment is based on conservative, health-protective assumptions that ensure that exposures to trinexapac-ethyl are not underestimated. These assumptions include tolerance-level residues and 100% crop treated estimates for all commodities. Furthermore, conservative, upper-bound assumptions were used to determine exposure through drinking water and residential sources, such that these exposures have not been underestimated.

4.4.1 Completeness of the Toxicology Database

The toxicology database for trinexapac-ethyl is complete for the risk assessment. There are acceptable studies available for toxicity endpoint selection. There are acceptable/guideline acute and subchronic neurotoxicity studies and an immunotoxicity study. The HASPOC determined, based the weight of the evidence (WOE), that the subchronic inhalation study could be waived (Dunbar, 2014, TXR # 0056699).

4.4.2 Evidence of Neurotoxicity

There was no evidence of neurotoxicity in the acute and subchronic neurotoxicity studies in rats at the limit dose. Nevertheless, dose-related neuropathology characterized as focal bilateral vacuolation of the dorsal medial hippocampus and/or lateral midbrain in both sexes was observed at study termination in the chronic dog study at the two highest doses tested (356 mg/kg/day: 3/8 dogs and 727 mg/kg/day: 8/8 dogs in the mid- and high dose groups, respectively). The vacuolation was associated with the astrocytes and oligodendrocytes. The lesions remained confined to the supporting cells in the central nervous system (CNS) and did not progress to more advanced or more extensive damage of the nervous tissue. The lesions were not associated with other neuropathological findings or overt neurological signs. The biological significance of these lesions is not known. Similar microscopic lesions in the brain were not

reported in the 90-day dog study, although, there was an inconsistency in the neuropathological screening of the brain in the 90-day study. Furthermore, similar lesions were not observed in the rat or mouse following subchronic or chronic dietary exposure, and there was no other evidence in any species tested to indicate a neurotoxicity potential.

4.4.3 Evidence of Sensitivity/Susceptibility in the Developing or Young Animal

Evidence of increased qualitative and/or quantitative susceptibility of the offspring was seen only at high doses in the developmental rat and rabbit studies, and in the rat reproduction study. Developmental toxicity in the rat was only observed at the limit dose (increased incidence of asymmetrical sternebrae at 1,000 mg/kg) in the absence of maternal toxicity. In the rabbit, no maternal toxicity was demonstrated at the highest dose tested [360 mg/kg/day], but there was a decrease in the mean number of fetuses/litter and an increase in post-implantation loss and early resorptions at this dose level. Therefore, clearly identified NOAELs were established in both the rat and rabbit developmental studies. Reproductive toxicity was not observed in the rat reproduction study, but decreased pup survival and decreased pup body weight/body weight gain during lactation were observed above the limit dose (1212 mg/kg/day) with only reduced body weight/body weight gain and food consumption observed in the parental animals at a comparable LOAEL (1212 mg/kg/day). The NOAEL for this study was 594 mg/kg/day for offspring and parental animals.

Although, there is evidence of susceptibility in the rat and rabbit developmental studies and qualitative susceptibility in the 2-generation rat reproduction study, these effects only occurred at the highest doses tested (360-1200 mg/kg/day) for each study, and there were clearly identified NOAELs/LOAELs for the rabbit developmental study (60/593 mg/kg/day), the rat developmental study (200/1000 mg/kg/day) and for the reproduction study (593/1212 mg/kg/day) for each fetal/offspring effect. Therefore, there are no residual concerns with respect to developmental and reproductive effects.

4.4.4 Residual Uncertainty in the Exposure Database

There are no residual uncertainties in the exposure database. Since the dietary and non-dietary exposure estimates were based on several conservative assumptions, HED does not believe the exposure assessment underestimates risk. These assumptions include tolerance-level residues and 100% crop treated estimates for all commodities. Actual exposures and risks from trinexapac-ethyl will likely be lower. Furthermore, conservative, upper-bound assumptions were used to determine exposure through drinking water and residential sources, such that these exposures have not been underestimated.

4. 5 Toxicity Endpoint and Point of Departure Selections

The details for selecting toxicity endpoints and points of departure (PoDs) are presented in

Appendix A2. Based on the existing and new use patterns proposed by the registrant for trinexapac-ethyl, the exposure is expected to be via the oral, dermal, and inhalation routes.

4.5.1 Dose-Response Assessment: The summary of toxicological doses and endpoints for trinexapac-ethyl that were used in the human health risk assessments is shown in Table 4.4.5.

Acute Dietary (General Population): No appropriate endpoint for the general population including infants and children was found since no effects attributable to a single dose were identified in the toxicology database.

Acute Dietary (Females 13-49 years of age): A rabbit developmental toxicity study was selected for the acute dietary endpoint for females 13-49 years old. The NOAEL was 60 mg/kg/day and the LOAEL was 360 mg/kg/day based on a decrease in the mean number of fetuses/litter and an increase in post-implantation loss and early resorptions. An Uncertainty Factor (UF) of 100 was applied to account for interspecies extrapolation (10X) and intraspecies variability (10X). The FQPA SF was reduced to 1X. Therefore, the acute reference dose (aRfD) for females 13-49 was calculated to be 0.6 mg/kg/day. This endpoint will be protective of the fetal effects (increased incidence of asymmetrical sternebrae) observed at higher doses (1000 mg/kg/day) in the rat. The selection of this study and the PoD is protective of the potentially pregnant female and the developing fetus. It is also a conservative endpoint since this is a large dose spread (the NOAEL is 6-fold lower than the LOAEL).

Chronic Dietary: Quantification of chronic dietary (dietary and drinking water) risks was performed using the chronic oral toxicity in dogs with a NOAEL of 31.6 mg/kg/day based on elevated serum cholesterol values in females, mucoid feces in females, bloody feces in both sexes, and minimal focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in both sexes at the LOAEL of 357 mg/kg/day. This endpoint is protective since the dog is the most sensitive species. Additionally, there is a large dose spread in the chronic dog study (the NOAEL is 10-fold lower than the LOAEL) but there is a clear NOAEL and LOAEL. Vacuolation was observed in all dogs at the high level (males 727 mg/kg/day; females 784 mg/kg/day) and was associated with the astrocytes and oligodendrocytes in the hippocampus. The lesions remained confined to the supporting cells in the CNS and did not progress to more advanced or more extensive damage of the nervous system. Similar lesions were not reported in the 90-day dog study but there is an inconsistency in the neuropathological screening of the brain, which precludes a definitive determination on the occurrence of this lesion following subchronic exposure. Similar lesions were not observed in the rat or mouse following acute, subchronic or chronic dietary exposure. Because of the concern for the brain lesions and the uncertainty of the timing of the brain lesions, the use of the dog sturdy is warranted in this case to be protective of these concerns.

Incidental Oral (Short-Term): An endpoint for the incidental oral exposure scenario for children was not identified from the available trinexapac-ethyl toxicity studies. The rationale for

this conclusion is based on the following weight of evidence considerations: 1) the toxicity observed during the appropriate duration of concern (short/intermediate term) were observed only at high doses [LOAEL = 900 mg/kg/day in the dog, 1000 mg/kg/day in the rat, and 1212 mg/kg/day in the reproduction study]; 2) the brain lesions observed in the chronic dog study are not appropriate for this scenario since the brain lesions were seen only at termination (i.e. after exposure to 1-year) and toxicity observed after chronic exposure is not appropriate for application to the short/intermediate term scenarios; 3) the brain lesion observed in the chronic study are not expected to occur after short term exposure due to the lack of similar lesions or associated toxicity (i.e., clinical neurologic signs) in the 90-day studies in rats or dogs; 4) the cause for concern for the brain lesions is low because of the minimal lesion severity, lack of associated changes (i.e., gliosis, myelin loss astrocyte hypertrophy, neuronal necrosis etc), and the non-specific nature of the lesions (i.e., focal vacuoles); 5) the overall toxicity profile of this chemical clearly shows that toxicity is observed only at high doses (lowest LOAEL = 360 m/k/d in the chronic dog study); and 6) the *in utero* endpoint identified in the rabbit study is not appropriate for this population of concern (children) (D392798, M. Hawkins, 11/23/2011).

Dermal and Inhalation (Short- and Intermediate-Terms): The rabbit developmental toxicity study was selected with a NOAEL of 60 mg/kg/day and a LOAEL of 360 mg/kg/day based on a decrease in mean number of fetuses/litter and an increase in post-implantation loss. This selected endpoints are only applicable to adults (females) because the endpoint is based on *in utero* effects. HED reviewed the full toxicological database for trinexapac-ethyl in an attempt to select endpoints for the dermal and inhalation children scenarios; however, no appropriate endpoint was available. Detailed rationales are described as follows.

For adults, the short- and intermediate-term dermal and inhalation scenarios are both based on the *in utero* effect observed in the rabbit developmental study (LOAEL 360 mg/kg/day). This is protective of female workers that may become pregnant while exposed to trinexapac-ethyl. The chronic dog study was also considered since it has a similar LOAEL (357 mg/kg/day). The NOAELs established in these two studies, however, are 2-fold different due to the dose-spread in the two studies. Therefore, the higher NOAEL of 60 mg/kg/day from the rabbit developmental study (LOAEL 360 mg/kg/day) is protective of the effects observed (LOAEL 357 mg/kg/day) in the chronic dog study with NOAEL of 31.6 mg/kg/day. Although the 21-day rabbit dermal study did not result in systemic toxicity at the limit dose and suggests a dermal assessment is not warranted, this study is performed only in adult animals, not pregnant animals. It is currently unknown if a dermal study with pregnant rabbits would result in an *in utero* effect similar to that observed in the rabbit developmental study. Therefore, the use of the rabbit developmental study for the short- and intermediate-term dermal and inhalation scenarios is conservative and protective for these routes of exposure.

For children, the short- and intermediate-term dermal and inhalation endpoints selected for adults are not applicable because the adult endpoints are based on *in utero* effects. HED reviewed the full toxicological database for trinexapac-ethyl in an attempt to select endpoints for children for

the dermal and inhalation exposure scenarios; however, no appropriate endpoints were identified for these scenarios based on the following reasons: 1) dermal irritation effects are mild after 21/28 days of exposure in the rabbit study; 2) dermal irritation effects after a single exposure disappear after seven days; 3) there are no systemic effects up to the limit dose in the dermal rabbit study; 4) there are no adverse toxicological effects (at doses close to the limit dose of 1000 mg/kg/day) in the 2-generation reproductive study in rats or in the chronic/carcinogenicity studies in rats and mice; 5) there is no maternal toxicity in the developmental toxicity studies in both rats and rabbits; 6) there are no identifiable endpoints for the oral studies in the rat or rabbit.

Table 4.4.5. Summary of Toxicological Doses and Endpoints for Trinexapac-ethyl for use in Human Health Risk Assessments.

Exposure/ Scenario	Point of Departure	Uncertainty/ FQPA Safety Factors	RfD, PAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (General Population, including Infants and Children)	No appropriate endpoint for the general population including infants and children was available.			
Acute Dietary (Females 13-49 years of age)	NOAEL = 60 mg/kg/day	UF_A= 10x UF_H = 10x FQPA SF= 1x	Acute RfD = 0.6 mg/kg aPAD = 0.6 mg/kg/day	**Developmental rabbit study** ***MRID 41869524*** LOAEL = 360 mg/kg, based on a decrease in mean number of fetuses/litter and an increase in post-implantation loss and early resorptions.
Chronic Dietary (All Populations)	NOAEL= 31.6 mg/kg/day	UF_A= 10x UF_H = 10x FQPA SF= 1x	Chronic RfD = 0.32 mg/kg/day cPAD = 0.32 mg/kg/day	**Chronic oral toxicity study – *dog*** ***MRID 42779402/ 42779401*** LOAEL = 357 mg/kg/day, based on elevated serum cholesterol values in females, mucoid feces in females and bloody feces in both sexes, and minimal, focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in both sexes
Incidental Oral (Short -Term)	No appropriate endpoint for the incidental oral scenario for children.			

Table 4.4.5. Summary of Toxicological Doses and Endpoints for Trinexapac-ethyl for use in Human Health Risk Assessments.

Exposure/ Scenario	Point of Departure	Uncertainty/ FQPA Safety Factors	RfD, PAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal & Inhalation Short and Intermediate- Term (Adults only)	NOAEL = 60 mg/kg/day Dermal absorption rate = 77.5% of oral absorption Inhalation rate = 100 % of oral absorption	UF_A= 10x UF_H = 10x FQPA SF= 1x	Residential LOC for MOE = 100 Occupational LOC for MOE = 100	**Developmental rabbit study** ***MRID 41869524*** LOAEL = 360 mg/kg, based on a decrease in mean number of fetuses/litter and an increase in post-implantation loss and early resorptions.
Dermal & Inhalation Short and Intermediate- Term (Children)	No appropriate endpoint for these exposure scenario for children.			
Cancer (oral, dermal, inhalation)	Classification: "Not likely to be Carcinogenic to Humans"			

Point of Departure (PoD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). FQPA SF = FQPA Safety Factor. PAD = population adjusted dose (a = acute, c = chronic). RfD = reference dose. MOE = margin of exposure. LOC = level of concern.

4.5.2 Recommendation for Combining Routes of Exposure for Risk Assessment

In accordance with the requirements of the FQPA (1996), HED has considered the potential for concurrent exposure to trinexapac-ethyl via oral, dermal, and inhalation routes. HED combines exposure from different routes for each population if the same toxic effects are seen for that duration of exposure by each route. There are no short-term oral, dermal or inhalation endpoints for children; therefore a discussion of combining routes is not relevant for this population subgroup. Since the short-term oral, dermal and inhalation PoDs for adults (females) were selected from a single study, exposure from these three routes of exposure should be combined for aggregate risk assessment.

4.5.3 Cancer Classification and Risk Assessment Recommendations

Trinexapac-ethyl is classified as "Not Likely to be Carcinogenic to Humans"; therefore, a cancer dietary exposure assessment was not conducted.

4.6 Endocrine Disruption

As required under FFDCA section 408(p), EPA has developed the Endocrine Disruptor Screening Program (EDSP) to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine related effects caused by the substance, and establish a dose-response relationship between the dose and the E, A, or T effect.

Between October 2009 and February 2010, EPA is issuing test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. This list of chemicals was selected based on the potential for human exposure through pathways such as food and water, residential activity, and certain post-application agricultural scenarios. This list should not be construed as a list of known or likely endocrine disruptors.

Trinexapac-ethyl is not among the group of 58 pesticide active ingredients on the initial list to be screened under the EDSP. Under FFDCA Sec. 408(p), the Agency must screen all pesticide chemicals. Accordingly, EPA anticipates issuing future EDSP test orders/data call-ins for all pesticide active ingredients.

For further information on the status of the EDSP, the policies and procedures, the list of 67 chemicals, the test guidelines and the Tier 1 screening battery, please visit our website: http://www.epa.gov/endo/.

5.0 Dietary Exposure and Risk Assessment

5.1 Residues of Concern Summary

For plants (primary and rotational crops) and livestock commodities, the Residues of Concern Knowledgebase Subcommittee (ROCKS) recommends that parent trinexapac-ethyl and the free acid metabolite, trinexapac (CGA 179500), are the residues of concern for both the tolerance expression and the risk assessment. For drinking water, the residue of concern are the parent ester including its free acid CGA-179500, CGA-313458, an open chain cyclohexane ring and an unidentified hydroproduct M3 (ROCKS memo, D390121, 7/12/11).

Table 5.1.4. Summary of Metabolites and Degradates of Trinexapac-ethyl.			
Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Plants	Primary Crop	Trinexapac-ethyl parent + Trinexapac acid (CGA 179500)	Trinexapac-ethyl parent + Trinexapac acid (CGA 179500)
	Rotational Crop		
Livestock	Ruminant		
	Poultry		
Drinking Water		Total residues (parent + identified degradates)[1]	Not Applicable
[1] A total major residue approach is recommended, including parent and major environmental degradates trinexapac (CGA-179500), 2-(4-cyclopropyl-4-hydroxy-2-oxobutyl)succinic acid (CGA-313458), the open chain cyclohexane ring, and the unidentified hydroproduct M3.			

5.2 Food Residue Profile

Available metabolism data shows that trinexapac-ethyl does translocate to the growing shoot when applied as a foliar application. Trinexapac-ethyl is used at relatively low application rates, and the submitted field trial studies demonstrate that when applied at post-emergence, quantifiable residues are seen in cereal grains treated up to 51-100 days before harvest. Also, data from the confined rotational crop study indicate that trinexapac-ethyl residues are detected (lettuce and wheat) at the 30-day plant back interval. Quantifiable residues were only seen in livestock at low levels in the animal feedstuff associated with this action.

5.3 Water Residue Profile

The drinking water residues used in the dietary risk assessment were provided by the Environmental Fate and Effects Division (EFED) in the following memorandum: "*Drinking Water Assessment for Trinexapac-Ethyl New Use on Rice and Rye*" (C. Koper, D421848, 10/01/14) and incorporated directly into the dietary assessment. A Tier I rice model for drinking water assessment was conducted to support the human health risk assessment for the new food use registration for trinexapac ethyl on rice and rye. A separate model run for rye is not required since application rates are the same for rye as previously assessed on cereal grains (D377936; 08-09-11); therefore, estimated drinking water concentrations from the rye use are expected to be the same as from the currently registered cereal grain uses. The recommended estimated drinking water concentrations (EDWCs) for the human health risk assessment are based on highest predicted values for surface water and ground water. For surface water, application (1 application at 0.045 lb ai/acre) to rice yielded the highest EDWCs. The acute and chronic concentrations were estimated to be 31.68 ppb. For ground water, based on the proposed highest annual use rate for turf (8 applications at 0.34 lb ai/acre), the PRZM-GW (v.1.07) model

estimated an acute concentration of 0.116 ppb and 30-year average (chronic) concentration of 0.054 ppb. HED notes that the rice model estimated surface water concentration is a calculated water number in the rice paddy water reflecting the concentration in the rice paddy at the time of application. Use of this number in a chronic assessment is considered highly conservative; however since there is no chronic refinement for the rice water and since risks are not of concern, that value was used in the chronic dietary assessment.

Water residues were incorporated in the DEEM-FCID into the food categories "water, direct, all sources" and "water, indirect, all sources." Table 5.3 provides a summary of the Tier 2 modeled drinking water concentrations. The model and its description are available at the EPA internet site: http://www.epa.gov/oppefed1/models/water/.

Table 5.3. Recommended Trinexapac-Ethyl Estimated Drinking Water Concentrations (EDWCs) for Surface Water and Ground Water based on selected Crop Scenarios

Proposed Label Use	Model	Method[1]	Maximum Application Rate (interval between applications)	1-in-10 year acute (µg/L)	1-in-10 year chronic (µg/L)	30- year average (µg/L)
Surface Water						
Rice	Tier 1 Rice Model	NA	1 app @ 0.045 lb a.i./acre	**31.68** *(12.61)*[2]	**31.68** *(1.56)*	**31.68** *(1.04)*
Ground Water						
Turf	PRZM-GW	G	8 app @ 0.34 lb a.i./acre	**0.116** *(0.009)*	NA	**0.054** *(0.009)*

[1] G = foliar, ground application, NA = Not Applicable.
[2] Italicized values generated from turf scenario (parent D377936; sub D395601)
Bold numbers denote maximum EDWC values. Italicized numbers denote maximum EDWC values from previous drinking water assessment (parent D377936; Sub D395601; 10-28-11).

5.4 Dietary Risk Assessment

5.4.1 Description of Residue Data Used in Dietary Assessment

Updated acute and chronic dietary (food and drinking water) exposure and risk assessments were conducting using the Dietary Exposure Evaluation Model software with the Food Commodity Intake Database (DEEM-FCID) Version 3.16. This software uses 2003-2008 food consumption data from the U.S. Department of Agriculture's (USDA's) National Health and Nutrition Examination Survey, What We Eat in America, (NHANES/WWEIA). The drinking water estimates provided by EFED are modeled estimates, and were generated using the most conservative model available for the existing uses. The unrefined acute and chronic assessment

using tolerance level residues for the dietary risk exposure analysis will not underestimate risk to the general U.S. population or any population subgroup (S. Biswas, 02/11/2015, D424893).

5.4.2 Percent Crop Treated Used in Dietary Assessment

Percent crop treated information was not used in these assessments. The acute and chronic assessments were both based on the assumption that 100% of all commodities with trinexapac-ethyl recommended tolerances will be treated.

5.4.3 Acute Dietary Risk Assessment

There were no appropriate toxicological effects attributable to a single exposure (dose) for the general population or any other population subgroups except females 13-49 years old; therefore, these population subgroups were not included in this assessment. For food and drinking water, the acute dietary risk estimate is below HED's level of concern (<100% of the aPAD) at the 95th percentile of exposure. The acute dietary exposure estimate for females 13-49 years old is 0.014667 mg/kg/day (2% of the aPAD).

5.4.4 Chronic Dietary Risk Assessment

Combined chronic dietary exposure estimates for food and drinking water are well below HED's level of concern. Using the DEEM-FCID software, dietary exposure is estimated at 0.008293 mg/kg/day for the U.S. population which is equivalent to 3% of the cPAD, and 0.020596 mg/kg/day, equivalent to 6% of the cPAD for children 1-2 years old, the population with highest estimated chronic dietary exposure to trinexapac-ethyl.

5.4.5 Cancer Dietary Risk Assessment

Trinexapac-ethyl is classified as "Not Likely to be Carcinogenic to Humans." Therefore, a cancer dietary exposure assessment was not conducted.

5.4.6 Dietary Summary Table

Table 5.4.6. Summary of Dietary (Food and Drinking Water) Exposure and Risk for Trinexapac-ethyl

Population Subgroup	Acute Dietary[1] (95th% Percentile)		Chronic Dietary		Cancer	
	Dietary Exposure (mg/kg/day)	% aPAD	Dietary Exposure (mg/kg/day)	% cPAD	Dietary Exposure (mg/kg/day)	Risk
General U.S. Population	N/A	N/A	0.008293	2.6	N/A	N/A
All Infants (< 1 year old)			0.008387	2.6		
Children 1-2 years old			**0.020596**	**6.4**		
Children 3-5 years old			0.019487	6.1		
Children 6-12 years old			0.013981	4.4		
Youth 13-19 years old			0.008238	2.6		
Adults 20-49 years old			0.006853	2.1		
Adults 50+ years old			0.005861	1.8		
Females 13-49 years old[1]	**0.014667**	**2.4**	0.006466	2.0		

[1] aPAD=0.6 mg/kg/day for females 13-49 only (no appropriate endpoint for general US population including infants and children) cPAD=0.32 mg/kg/day. Not likely to be carcinogenic to humans.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

As was mentioned, no new residential uses are being proposed at this time so an updated residential exposure assessment is not required; however, there are registered residential uses for trinexapac-ethyl that were assessed previously for turf using the 2012 Residential SOPs (M. Hawkins, D413030, 9/18/2013). The residential handler MOEs range from 240 to 21,000,000.

The residential post-application MOEs range from 450 to 61,000. Recommendations for the residential input of the aggregate remain unchanged from the previous memo. They are as follows:

- The recommended residential exposure estimate for use in the adult (female) aggregate assessment reflects dermal and inhalation exposure from handler exposure to sprays that are applied on turf with backpack sprayers (edging/banding), which resulted in a MOE of 240.
- The recommended residential exposure estimate for use in the child (11 – 16 years old) aggregate assessment reflects dermal exposure from mowing turf that has been treated with a granular formulation, which resulted in a MOE of 13,000.
- There are no endpoints for short-term oral, dermal or inhalation exposure for young children (< 11 years old); therefore quantitative residential exposure assessments are not conducted for younger children

The residential exposure/risk estimates and recommendations for the trinexapac-ethyl aggregate are provided below in Table 6.1.

Table 6.1. Recommendations for the Residential Exposures for the Trinexapac-ethyl Aggregate Assessment.[1]

Lifestage	Residential Handler						Residential Post-application							
	Dose (mg/kg/day)[2]			MOE[3]			Dose (mg/kg/day)[4]				MOE[5]			
	Dermal	Inhalation	Total	Dermal	Inhalation	Total	Dermal	Inhalation	Oral	Total	Dermal	Inhalation	Oral	Total
Short-Term														
Adult Female	0.2482	0.00034	0.249	240	170,000	**240[1]**	0.135	N/A	N/A	0.135	450	N/A	N/A	450
Child 11 to 16 years old	N/A	N/A	N/A	N/A	N/A	N/A	0.005	N/A	N/A	0.005	13,000	N/A	N/A	**13,000[1]**

1 Bolded risk estimates should contribute to the residential exposure portion of the aggregate assessment.
2 Residential Handler Dose = the highest handler dose for each applicable lifestage of all scenarios assessed from Table 6.1.1. (D413570) Total = dermal + inhalation.
3 Residential Handler MOE = the MOEs associated with the highest doses identified in Table 6.1.1. (D413570) Total = 1 ÷ ((1/Dermal MOE) + (1/Inhalation MOE)).
4 Residential Post-application Dose = the highest post-application dose for each applicable lifestage of all scenarios assessed from Table 6.2.1. (D413570) Total = dermal + inhalation + incidental oral, where applicable.
5 Residential Post-application MOE = the MOEs associated with the highest doses identified in Table 6.2.1 (D413570) Total = 1 ÷ (1/Dermal MOE) + (1/Inhalation MOE) + (1/Incidental oral MOE).

6.1 Spray Drift

Spray drift is a potential source of exposure to those nearby pesticide applications. This is particularly the case with aerial application, but, to a lesser extent, spray drift can also be a potential source of exposure from the ground application methods (e.g., groundboom and airblast) employed for trinexapac-ethyl. The agency has been working with the Spray Drift Task Force (a task force composed of various registrants which was developed as a result of a Data

Call-In issued by EPA), EPA Regional Offices and State Lead Agencies for pesticide regulation and other parties to develop the best spray drift management practices (see the agency's Spray Drift website for more information).[1] The agency has also developed a policy on how to appropriately consider spray drift as a potential source of exposure in risk assessments for pesticides. The potential for spray drift was quantitatively evaluated for trinexapac-ethyl during the *Registration Review* process (D413030).

HED notes that the proposed new uses on rice and rye do not impact the spray drift assessment.

6.2 Residential Bystander Post-Application Inhalation Exposure

Volatilization of pesticides may be a source of post-application inhalation exposure to individuals nearby pesticide applications. The Agency sought expert advice and input on issues related to volatilization of pesticides from its Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel (SAP) in December 2009, and received the SAP's final report on March 2, 2010 (http://www.epa.gov/scipoly/SAP/meetings/2009/120109meeting.html). The Agency has evaluated the SAP report and has developed a Volatilization Screening Tool and a subsequent Volatilization Screening Analysis (http://www.regulations.gov/#!docketDetail; D=EPA -HQ-OPP-2014-0219). During Registration Review, the Agency will utilize this analysis to determine if data (i.e., flux studies, route-specific inhalation toxicological studies) or further analysis is required for trinexapac-ethyl.

7.0 Aggregate Exposure/Risk Characterization

In accordance with the FQPA, HED must consider and aggregate (add) pesticide exposures and risks from three major sources: food, drinking water, and residential exposures. In an aggregate assessment, exposures from relevant sources are added together and compared to quantitative estimates of hazard (e.g., a NOAEL or PAD), or the risks themselves can be aggregated. When aggregating exposures and risks from various sources, HED considers both the route and duration of exposure.

7.1 Acute Aggregate Risk

Acute aggregate risk results from exposure to residues in food and drinking water alone. The acute dietary exposure analysis for females 13-49 years included both food and drinking water. Therefore, acute aggregate risk is equivalent to the acute dietary risk, as discussed in Section 5.4.3, above. All risk estimates are below HED's level of concern. No appropriate endpoint for the general population, including infants and children was found; therefore, aggregate assessments are not required for these population subgroups.

[1] Available: http://www.epa.gov/opp00001/factsheets/spraydrift.htm

7.2 Short- and Intermediate-Term Aggregate Risk

The short- and intermediate-term toxicological endpoints for trinexapac-ethyl are the same for each route of exposure. Therefore, for residential exposure scenarios, only short-term exposures were assessed, and are considered to be protective of intermediate-term exposure and risk. Exposures from these scenarios are aggregated with average dietary exposure from food and water which is considered to be background exposure. The LOCs for these exposure routes are all 100.

For the adult short- and intermediate-term aggregate risk assessment, chronic dietary exposure was added directly to the adult and children (11< 16 years old) post-application exposure estimate. To be health protective, the post-application scenario was used in the aggregate because it resulted in higher exposure than the handler scenario. The chronic dietary exposure estimate for females 13-49 years old and youth 13-19 years old were used to determine the aggregate risk estimate because they best match the lifestages assessed in the residential assessment.

Short-term aggregate margins of exposure for adult and children exceed HED's level of concern of 100 and are not of concern (MOEs $\geq$ 100). Short- and intermediate-term estimated exposure and risks are shown below in Table 7.2.

Table 7.2. Short-Term and/or Intermediate Term Dermal Aggregate Risk Calculation

	Short- or Intermediate-Term Scenario						
Population	**NOAEL mg/kg/day**	**LOC[1]**	**Max Allowable Exposure mg/kg/day[2]**	**Average Food and Water Exposure mg/kg/day[3]**	**Residential Post-application Dermal Exposure mg/kg/day[4]**	**Total Exposure mg/kg/day[5]**	**Aggregate MOE (food, water, and residential)[6]**
Child 11- 16 years old	60	100	0.60	0.008283	0.005	0.013283	4500
Adult Female				0.006466	0.249	0.255466	230

[1] An UF of 100x was applied to account for interspecies extrapolation (10x) and intraspecies variation (10x) and no additional uncertainty factors/safety factors are required.
[2] Maximum Allowable Exposure (mg/kg/day) = NOAEL/ (60 mg/kg/day)/LOC (100)
[3] Average food and water exposure from chronic dietary exposure for youth 13-19 yrs old and females 13-49 yrs old. See Table 5.4.6
[4]Residential Exposure = Dermal exposure from treated turf (See Table 6.1 and further details see Table 6.1.1. (D413570)
[5] Total Exposure = Avg Food & Water Exposure + Residential Exposure
[6] Aggregate MOE = NOAEL/Total Exposure

7.3 Cancer Aggregate Risk

The Cancer Assessment Review Committee (CARC) classified trinexapac-ethyl as "not likely to be carcinogenic to humans." Therefore, cancer aggregate cancer risk was not quantitatively assessed.

8.0 Cumulative Exposure/Risk Characterization

The Food Quality Protection Act (FQPA) requires the Agency to consider the cumulative risks of chemicals sharing a common mechanism of toxicity. The Agency determined that there was insufficient evidence to suggest trinexapac-ethyl shares a common mechanism of toxicity with other chemical substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Program concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/ cumulative/.

9.0 Occupational Exposure/Risk Pathway

9.1 Occupational Handler Exposure/Risk Estimates

In the previous occupational exposure assessment for trinexapac-ethyl (D413570), occupational handler and post-application exposures were assessed for the currently registered use for each trinexapac-ethyl crop, including cereal grains which was assessed at a maximum application rate of 0.11 lb ai/A. The maximum single application rates for both rice (0.045 lb ai/A) and rye (0.11 lb ai/A) are either the same or lower than crops that were previously assessed. Therefore, an updated occupational exposure assessment for the proposed new uses is not required at this time. The occupational handler assessment MOEs range from 140 to 2,100,000 with baseline attire and/or with the addition of gloves (as required on the labels). The occupational post-application MOEs on the day of application range from 87 to 43,000. Based on HED's occupational post-application exposure assessment, the REI for hand-harvesting sugarcane was estimated to be 2 days for short- and intermediate-term exposure. However, HED does not recommend that sugarcane REI's be changed on the trinexapac-ethyl labels at this time based on two main issues. One, almost all sugarcane harvesting in the United States is done mechanically. Two, the proposed trinexapac-ethyl labels have a 28-day PHI for sugarcane, so hand harvesting should not occur until after 28 days which is protective of the estimated REIs (M. Hawkins, 9/18/13, D413030).

Trinexapac-ethyl is classified as Toxicity Category III for acute oral, dermal, and eye irritation, and IV for acute inhalation and skin irritation. It is not a dermal sensitizer. Therefore, the acute toxicity categories for this chemical require a 12 hour restricted entry interval (REI) under 40 CFR 156.208 (c) (2) (iii)

10.0 Review of Incident Report

Trinexapac-ethyl incidents were analyzed from January 1, 2010- January 20, 2015 (S.Recore, 2/4/2015, D425401). There was 1 incidents identified from the Main IDS (Incident Data System) and 2 additional incidents reported involving more than one chemical; and 3 incidents identified in Aggregate IDS. Based on the low frequency and severity of incident cases reported for trinexapac-ethyl in both IDS and NIOSH SENSOR-Pesticides, there does not appear to be a concern at this time that would warrant further investigation. The Agency will continue to monitor the incident information and if a concern is triggered, additional analysis will be conducted.

11.0 References

DP#: 413570
Subject: Trinexapac-ethyl: Human Health Risk Assessment for Registration Review.
From: S. Piper, D. Smegal, and M. Hawkins
To: K. Keller
Date: 09/12/13
MRIDs: NA

DP#: 424894
Subject: Trinexapac-ethyl: Section 3 Registration Request to Add New Uses on Rice and Rye. Summary of Analytical Chemistry and Residue Data.
From: S. Bose Biswas
To: B. Benbow and K. Montague
Date: 2/11/2015
MRIDs: 49282807

DP#: 424893
Subject: Trinexapac-ethyl**:** Acute and Chronic Aggregate Dietary (Food and Drinking Water) Exposure and Risk Assessments to Support New Uses on Rice and Rye.
From: S. Bose Biswas
To: B. Benbow and K. Montague
Date: 2/11/2015
MRIDs: NA

DP#: 421848
Subject: Drinking Water Assessment for Trinexapac-ethyl New Use on Rice and Rye.
From: C. Koper, S. Sankula, M. Radtke
To: B. Benbow and K. Montague
Date: 10/01/14
MRIDs: NA

DP#: 413030
Subject: Preliminary Occupational and Residential Exposure/Risk Assessment for Registration Review.
From: M. Hawkins
To: S. Piper and K. Keller
Date: 09/18/13
MRIDs: NA

DP#: 425401
Subject: Trinexapac-ethyl: Tier I Review of Human Incidents for Preliminary Risk Assessment
From: S. Recore and E. Evans
To: S. Piper and A. Jakob
Date: 02/04/2015
MRIDs: NA

Toxicology:

41563920 Chau, R. Y.; McCormick, G. C.; and Arthur, A. T. (1989). 13-Week Oral Feeding Study in Dogs.
41563921 Chau, R. Y.; McCormick, G. C.; and Arthur, A. T. (1989). 13-Week Oral Feeding Study in Rats.
41563922 Huber, K. R.; Batastini, G.; and Arthur, A. T. (1989). 21-Week Dermal Toxicity Study in Rabbits.
41563923 Schoch, M. (1988). Developmental Toxicity (Teratogenicity) Study with CGA 163935 Technical in Rats.
41869524 Hughes, E. (1990). Developmental Toxicity (Teratogenicity) Study with CGA 163935 Technical in Rabbits.
42238104 Giknis, M. L.; Batastini, G.; and Arthur, A. T. (1992).52/104 Week Oral Toxicity Study in Rats.
42779401 Hardisty, J. F. (1992). Supplement to 52-Week Feeding Study in Dogs.

42779402 Chau, R. Y.; Kirchner, F. R.; and Arthur, A. T. (1991). 52-Week Feeding Study in Dogs.
43128603 Rudzki, M. W.; Batastini, G., and Arthur, A. T. (1991). 78-Week Oral Carcinogenicity Study in Mice.
43128604 Singh, A.; Hazelette, J.; and Yau, E. (1991). CGA-163935 Technical. A Two-Generation Reproductive Toxicity Study in Rats.
43128605 Geleick, D. Gene Mutations Test Mouse Lymphoma Mutagenicity Assay *in vitro*.
46809308 Deparade, E. (2001). CGA275537 Technical (metabolite of trinexapac-ethyl).
48764506 Beck, M. (2012). Trinexapac-Ethyl- An Oral (Gavage) Acute Neurotoxicity Study in Rats Final Report.
48764507 Beck, M. (2012). Trinexapac-ethyl – Subchronic (13-week) Dietary Neurotoxicity Study in Rats.

Appendix A: Toxicology Profile and Executive Summaries

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for a food use for trinexapac-ethyl are in Table A.1 Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used. The toxicity profiles are present below in Tables A.2.1 and A.1.2.

Table A.1. Toxicology Data Requirements

Test	Technical	
	Required	**Satisfied**
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (rodent)	yes	yes
870.3150 Oral Subchronic (nonrodent)	yes	yes
870.3200 21/28-Day Dermal	yes	yes
870.3250 90-Day Dermal	no	---
870.3465 90-Day Inhalation	yes	---[a]
870.3700a Developmental Toxicity (rodent)	yes	yes (83-3a)
870.3700b Developmental Toxicity (nonrodent)	yes	yes (83-3b)
870-3800 Reproduction	yes	yes (83-4)
870.4100a Chronic Toxicity (rodent)	yes	yes
870.4100b Chronic Toxicity (nonrodent)	yes	yes
870.4200a Oncogenicity (rat)	yes	yes
870.4200b Oncogenicity (mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes

Table A.1. Toxicology Data Requirements		
Test	**Technical**	
	Required	**Satisfied**
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5375 Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5395 Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a Acute Delayed Neurotox. (hen)	no	---
870.6100b 90-Day Neurotoxicity (hen)	no	---
870.6200a Acute Neurotox. Screening Battery (rat)	yes	yes
870.6200b 90 Day Neurotox. Screening Battery (rat)	yes	yes
870.6300 Develop. Neurotoxicity	CR	no
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	no	yes
870.7800 Immunotoxicity	yes	yes
Non guideline Thyroid MOA Investigation Study	No	---

[a]Data waiver granted by HASPOC, July 11, 2013 (TXR 0056699).

A.2.1 Toxicity Profiles

Table A.2.1. Acute Toxicity Profile of Trinexapac-ethyl (97%).

Guideline No.	Study Type	MRID	Results	Toxicity Category
870.1100	Acute oral [rat]	41563901	♂ LD_{50} = 4613 mg/kg ♀ LD_{50} = 4212 mg/kg Combined LD_{50} = 4458 mg/kg	III
870.1200	Acute dermal [rabbit]	41563910	LD_{50} > 4000 mg/kg	III
870.1300	Acute inhalation [rat]	41563912	$LC_{50} \geq 5.3$ mg/L	IV
870.2400	Acute eye irritation [rabbit]	41563914	Minimal irritant; cleared by 72 hours	III
870.2500	Acute dermal irritation [rabbit]	41563916	Slightly irritating; cleared by day 7	IV
870.2600	Skin sensitization [guinea pig]	41869522	Not a dermal sensitizer	N/A

Table A.2.2 Acute Toxicity Profile- – Vision™ Formulation (23% Trinexapac-ethyl)

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral rat]	41563909	LD_{50} = 4514 mg/kg (both sexes)	III
870.1200	Acute dermal [rabbit]	41563911	LD_{50} >2020 mg/kg	III
870.1300	Acute inhalation [rat]	41563913	LC_{50} = > 0.912 mg/L	III
870.2400	Acute eye irritation [rabbit]	41563915	severe irritant	I
870.2500	Acute dermal irritation [rabbit]	41563916	moderate irritant	III
870.2600	Skin sensitization [guinea pig]	41869519 41869521	not a dermal sensitizer	N/A

Table A.2.3 Acute Toxicity Profile – Primo™ Formulation (12% Trinexapac-ethyl)

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral rat]	41869514	LD_{50} = 5010 mg/kg (males) LD_{50} = 5730 mg/kg (females)	IV
870.1200	Acute dermal [rabbit]	41869515	LD_{50} >2020 mg/kg	III
870.1300	Acute inhalation [rat]	41869516	LC_{50} = > 0.888 mg/L	III
870.2400	Acute eye irritation [rabbit]	41869517	moderately irritating; corneal involvement, conjunctival irritation did not clear within 7 days	II
870.2500	Acute dermal irritation [rabbit]	41869519	slightly irritating	IV
870.2600	Skin sensitization [guinea pig]	41869522	not a dermal sensitizer	N/A

Table A.2.4. Subchronic, Chronic and Other Toxicity Profile of Trinexapac-ethyl.

Guideline No./ Study Type	MRID No. (year) Classification /Doses	Results
870.3100 90-Day oral toxicity [rat]	MRID 41563921 (1989) 0, 5, 50, 500, 5000, 20,000 ppm [males 0, 3, 34, 346, 1350 mg/kg/day] [females 0, 4, 38, 395, 1551 mg/kg/day]acceptable/guideline	NOAEL = 20,000 ppm [males 1350/females 1551 mg/kg/day HDT
870.3150 13-week oral toxicity in nonrodent (dog)	MRID 41563920 (1989) 0, 50, 100, 15,000, 30,000 ppm [males 0, 2.0, 34.9, 515.9, 927.1 mg/kg/day] [females 0, 1.9, 38.8, 582.4, 890.8 mg/kg/day]	NOAEL = 15,000 ppm [males 515.9/females 582.4 mg/kg/day LOAEL = 30,000 ppm [males 927.1/females 890.8 mg/kg/day, based on clinical signs (few feces and emaciation) decreased BWG/FC/FE in both sexes (related to lack of palatability) and diffuse thymic atrophy
7-week pilot study	acceptable/guideline MRID 41869523 0, 500, 5000, 15,000-50,000 ppm [males 0, 22, 219, (686, 956, 734)* mg/kg/day] [females 0, 23, 214, (680, 1373, 965)* mg/kg/day] *15,000 ppm (days 1-3); 30,000 ppm (days 4-28); 50,000 ppm (weeks 4-7)	Negative BWG in males HDT from week 5 on; HDT females from week 6 on; BW of HDT males 81% of control/females 74% control at week 7; severe decrease in food consumption HDT; tubular dilatation and degeneration/regeneration of epithelial cells of renal tubules at HDT; diffuse thymic atrophy at mid- and high-dose females and high-dose males.
870.3200 21/28-Day dermal toxicity (rabbit)	MRID 41563922 (1989) [46809310 (2006)] 0, 10, 100, or 1000 mg/kg/day,	**Systemic toxicity** NOAEL: 1000 mg/kg/day; LOAEL: Not determined **Local dermal irritation**

Table A.2.4. Subchronic, Chronic and Other Toxicity Profile of Trinexapac-ethyl.		
Guideline No./ Study Type	**MRID No. (year) Classification /Doses**	**Results**
	acceptable/guideline	NOAEL: 10 mg/kg/d; LOAEL: 100 mg/kg/d, based on hyperkeratosis and subacute lymphocytic infiltrates in the skin.
870.3365 28-Day inhalation toxicity (rat)	Data waiver granted by HASPOC July 11, 2013 TXR 0056699.	
83-3a Prenatal developmental in rodent [rat]	MRID 41563923 (1988) 0, 20, 200, 1000 mg/kg/day gestation days 6-15 Acceptable/guideline; (does not fulfill 870.3700a because study pre-dates this 1998 guideline)	**Maternal** NOAEL = 1000 mg/kg/day, highest dose tested **Developmental** NOAEL = 200 mg/kg/day **Developmental** LOAEL = 1000 mg/kg/day, based on increased incidence of asymmetrically-shaped sternebrae.
83-3b Prenatal developmental in nonrodent (rabbit)	MRID 41869524 (1990) 0, 10, 60, or 360 mg/kg/day gestation days 7-19 Acceptable/guideline;	**Maternal toxicity NOAEL** = 360 mg/kg/day, highest dose tested **Developmental toxicity NOAEL** = 60 mg/kg/day **Developmental toxicity LOAEL** = 360 mg//kg/day, based on a decrease in the mean number of fetuses/litter and an increase in post-implantation loss and early resorptions[a]
83-4 Reproduction and fertility effects (rats)	MRID 43128604 (1991) 0, 10, 1000, 10,000, 20,000 ppm [P0 males: 0, 0.59, 59.97, 595.26, 1169.16 mg/kg/day] [P0 females: 0, 0.75, 74.84, 736.89, 1410.08 mg/kg/day] F1 males: 0, 0.59, 59.10, 591.76, 1254.96 mg/kg/day] F1 females 0, 0.77, 77.17, 765.20, 1559.65 mg/kg/day] Acceptable/guideline	**Parental toxicity NOAEL** = 10,000 ppm [males 593.5/females 751.1 mg/kg/day] **Parental toxicity LOAEL** 20,000 ppm [males 1212.1/females 1484.9 mg/kg/day], based on reduced premating and gestation body weight/body-weight gain and food consumption **Reproductive NOAEL** = 20,000 ppm [males 1212/females 1484 mg/kg/day]. No adverse treatment-related effect on reproductive parameters up to and including 20,000 ppm (HDT) **Offspring NOAEL** = 10,000 ppm [males 593.5/females 751.1 mg/kg/day] **Offspring LOAEL** = 20,000 ppm [males 1212.1/females 1484.9 mg/kg/day], based on decreased F1 postnatal survival and reduced pup body weights in both generations [both sexes].
870.4100a Chronic toxicity rodents (rat)	MRID 42238104 (1992) 0, 10, 100, 3000, 10,000, 20,000 ppm M 0, 0.38, 3.87, 115.6, 392.7, 805.7 mg/kg/day F 0, 0.49, 4.88, 147.4, 494.0, 1054 mg/kg/day acceptable/guideline	**Systemic toxicity NOAEL =** 20,000 ppm [males 806/females 1054 mg/kg/day, highest dose tested.

Table A.2.4. Subchronic, Chronic and Other Toxicity Profile of Trinexapac-ethyl.		
Guideline No./ Study Type	**MRID No. (year) Classification /Doses**	**Results**
870.4100b Chronic toxicity nonrodent (dogs)	MRID 42779402/42779401 (1991-92) 0, 40, 1000, 10,000, or 20,000 ppm [males 0, 1.56, 31.62, 356.72, or 726.65 mg/kg/day] [females 0, 1.37, 39.54, 357.13, 783.83 mg/kg/day] acceptable/guideline	**Systemic toxicity NOAEL** = 1000 ppm [males 31.62/females 39.54 mg/kg/day **Systemic toxicity LOAEL** = 10,000 ppm [males 365.72/females 357.13 mg/kg/day], based on elevated serum cholesterol values in females, mucoid feces in females and bloody feces in both sexes, and minimal, focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in both sexes.
870.4200 Carcinogenicity (rat) Sprague-Dawley	MRID 42238104 (1992) 0, 10, 100, 3000, 10,000, 20,000 ppm M 0, 0.38, 3.87, 115.6, 392.7, 805.7 mg/kg/day F 0, 0.49, 4.88, 147.4, 494.0, 1054 mg/kg/day acceptable/guideline	See above under 870.4100a There was a possible treatment related increased incidence of squamous cell carcinoma of the forestomach in M at 20000 ppm (HDT); however, this is not considered toxicologically relevant to humans. No treatment-related difference detected in total number of animals with tumors or in the total number of benign or malignant tumors at 52 or 104 weeks. No treatment-related effect on the time-dependent occurrence of tumor-bearing animals. **Not Likely to be Carcinogenic to Humans**
870.4300 Carcinogenicity (mouse) CD-1 [Crl:CD-1 (ICR)Br]	MRID 43128603 (1991) 0, 7, 70, 1000, 3500, 7000 ppm [males 0, 0.91, 9.01, 130.81, 450.72, 911.77 mg/kg/day] [females 0, 1.08, 10.66, 154.08, 538.73, 1073.42 mg/kg/day] acceptable/guideline	**Systemic toxicity NOAEL** = 7000 ppm [males 911/females 1073 mg/kg/day], the highest dose tested. There was no treatment-related increase in tumors of any type in either sex at dose levels up to an including 7000 ppm, the HDT **No evidence of carcinogenicity**
870.5100 Bacterial Reverse Gene Mutation Assay	46809308 (2001) *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 *Escherichia coli* strain WP2*uvr*A 0, 312.5, 625, 1250, 2500, or 5000 µg/plate ± S9 metabolic activation Acceptable/guideline	**Negative** up to the limit concentration.
870.5300 Mouse Lymphoma Cells/Mammalian	43128605 (1993) Mouse lymphomaL5178Y cells (at the thymidine kinase locus) 0, 7.54, 30.16, 120.62, or	**Negative** up to a precipitating concentration (1930 µg/mL)

Table A.2.4. Subchronic, Chronic and Other Toxicity Profile of Trinexapac-ethyl.		
Guideline No./ Study Type	**MRID No. (year) Classification /Doses**	**Results**
Activation Gene Forward Mutation Assay at $TK^{+/-}$ locus	1930 µg/mL for 4 hours ± S9 metabolic activation Acceptable/guideline	
870.5395 Structural chromosomal aberration test - Micronucleus Test Mouse	41563926 (1989) 42081402 (1991) 41869527 (1991) M and F mouse bone marrow cells (erythrocytes) 0, 1000, 2000, or 4000 mg/kg bw (sacrifice at 16, 24, and 48 h) Initial assay: 0 or 3000 mg/kg bw (sacrifice at 16, 24, and 48 h) Confirmatory assay: 0, 750, 1500, or 3000 mg/kg bw (sacrifice at 48 h) Acceptable/guideline	**Negative** up to doses in excess of the limit dose. Significant increased frequency of micronucleated polychromatic erythrocytes in M and sexes combined at 48 h in the initial assay; however, values were within historical control range and not observed in the confirmatory assay at 3000 mg/kg bw at 48 h . In this study possible weak clastogen, however, weight of evidence suggestsCGA-163935 not likely clastogenic.
870.5550 Other Genotoxicity *In vitro* UDS in Primary Rat Hepatocytes	41604205 (1987) 41869528 (1991) Preliminary cytotoxicity assay: 0, 5, 10, 21, 41, 82, 164, 328, 656, 1313, 2625, or 5250 µg/mL Initial UDS assay: 0, 0.8, 4, 20, 100, 200, or 400 µg/mL; Confirmatory UDS assay: 0, 4, 20, 100, 150, 200, 300, 400, or 500 µg/mL Acceptable/guideline	**Negative** up to a cytotoxic concentrations
870.6200a Acute neurotoxicity screening battery (rat)	48764506 (2012) 0, 500, 1000, or 2000 mg/kg (♂♀) Acceptable/guideline	**Systemic/Neurotoxicity** NOAEL = >2000 mg/kg (Highest dose tested, limit dose) LOAEL was not established
870.6200b 90-day neurotoxicity screening battery (rat)	48764507 (2012) 0, 3750, 7500, or 15,000 ppm (0, 233, 463, or 948 mg/kg/day, ♂ 0, 294, 588, or 1171 mg/kg/day, ♀)	**Systemic/Neurotoxicity** NOAEL = >15,000 ppm (Highest dose tested; near or in excess of the limit dose) LOAEL was not established.
870.7485 Metabolism and pharmacokinetics (rat)	MRID 41563927 (1990) i. v. 0.91 mg/kg [^{14}C- CGA-163935] oral 0.97 or 166 mg/kg [^{14}C- CGA-163935]	Rapidly, extensively absorbed (both sexes) w/ >95% of administered dose being absorbed; little potential for accumulation; >85% eliminated w/in 12 hours via urine; 2% via feces w/in 24 hours; very little or no biliary excretion; no sex difference; free acid derivative

Table A.2.4. Subchronic, Chronic and Other Toxicity Profile of Trinexapac-ethyl.		
Guideline No./ Study Type	**MRID No. (year) Classification /Doses**	**Results**
	oral 0.97 mg/kg/day [CGA-163935] for 14 days followed by 0.97 mg/kg [^{14}C- CGA-163935] acceptable/guideline	resulting from hydrolysis of the ester bond of parent compound is major component in urine and feces; only other component was parent, found only in feces.
870.7600 Dermal penetration (rat)	MRID 42238105 (1990) 0, 0.01, 0.1, or 1.0 mg/cm^2 [^{14}C-CGA-163935] single dermal dose acceptable/guideline	Recovery of applied dose 97%-117%; most recovered in skin washes and urine; <1% in blood and feces; excreted in urine within 2 hours of dose 56.5% absorbed, with 21% associated with application site Dermal absorption factor = 77.5% based on 10 hour exposure regimen.
870.7800 Immunotoxicity study (female mice)	MRID 48444101 (2011) 0, 500, 2000, and 5000 ppm 0,160.2, 613.7, 1530.5 mg/kg/day acceptable/guideline	**Systemic toxicity NOAEL** = 5000 ppm (1530.5 mg/kg/day, the highest dose tested) the **LOAEL** was not established. **The immunotoxicity NOAEL for anti-SRBC AFC response and NK cell activity is 5000 ppm (1530.5 mg/kg/day), the LOAEL was not established.** Highest dose tested was in excess of the limit dose

A.3 Hazard Identification and Endpoint Selection

A.3.1 Acute Reference Dose (aRfD) - Females age 13-49

Study Selected: Prenatal Developmental Toxicity Study– rabbit
MRID No.: 41869524
Summary: See Appendix A, Guideline 870.3700a
Dose and Endpoint for Risk Assessment: NOAEL = 60 mg/kg, based on a decrease in the mean number of fetus/litter and an increase in post-implantation loss and early resorptions at the developmental toxicity LOAEL of 360 mg/kg/day.
Uncertainty Factor(s): 100X [10 interspecies; 10X intraspecies] FQPA SF = 1X, UF_{DB} = 1X.
Comments about Study/Endpoint/Uncertainty Factor: The route and duration of exposure are appropriate for selection of the acute dietary endpoint for females 13-49 years old. The toxicity could occur following a single exposure and is appropriate for this exposure scenario. This endpoint will also be protective of the **rat** fetal effects [increased incidence of asymmetrical sternebrae] observed at higher dose levels [1000 mg/kg/day]. This is a conservative endpoint since this is such a large dose spread (the NOAEL is 6-fold lower than LOAEL).

$$aRfD = aPAD = \frac{60\ mg/kg/day}{100} = 0.60\ mg/kg/day$$

A.3.2 Acute Reference Dose (aRfD) general population

An acute reference dose was not defined; no effects attributable to a single dose were identified

in the toxicology database. An *in utero* effect from the developmental rabbit study provided an acute reference dose for females 13-49 years old but is not appropriate for the general population.

A.3.3 Chronic Reference Dose (cRfD)

Study Selected: chronic oral toxicity – dog

MRID No.: 42779402/42779401

Executive Summary: See Appendix A, Guideline 870.4100 b

Dose and Endpoint for Risk Assessment: NOAEL = 1000 ppm [males 31.6 mg/kg/day; females 39.5 mg/kg/day], based on elevated serum cholesterol values in females, mucoid feces in females and bloody feces in both sexes, and minimal, focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in both sexes at the LOAEL of 10000 ppm [males 365.72/females 357.13 mg/kg/day].

Uncertainty Factor(s): 100X [10 interspecies; 10X intraspecies] FQPA SF = 1X, UF_{DB} = 1X.

Comments about Study/Endpoint/Uncertainty Factor: The chronic toxicity study in the dog (MRID 42779402/42779401) was selected for the chronic dietary cRfD because the route and duration of exposure are appropriate for selection of the chronic dietary endpoint. This endpoint is protective since the dog is the most sensitive species, there is a large dose spread in the chronic dog study (the NOAEL is 10-fold lower than the LOAEL), and there is a clear NOAEL and LOAEL. The vacuolation was also observed in all of the dogs at the high dose level (males 727 mg/kg/day /females 784 mg/kg/day) and was associated with the astrocytes and oligodendrocytes in the hippocampus. The lesions remained confined to the supporting cells in the CNS and did not progress to more advanced or more extensive damage of the nervous tissue. The lesions were not associated with other neuropathological findings or overt neurological signs. Similar microscopic lesions in the brain were not reported in the 90-day dog study, but there was an inconsistency in the neuropathological screening of the brain, which precludes a definitive determination on the occurrence of this lesion following subchronic exposure. Similar lesions were not observed in the rat (including neonates) or mouse following subchronic or chronic dietary exposure, and there was no other evidence in any other species tested to indicate a neurotoxicity potential.

$$\text{cRfD} = \text{cPAD} = \frac{31.6 \text{ mg/kg/day}}{100} = 0.32 \text{ mg/kg/day}$$

A.3.4 Incidental Oral Exposure (Short- and Intermediate-Term)

An endpoint for children for the incidental oral exposure scenario was also not identified from the available trinexapac-ethyl toxicity studies and the rationale for this conclusion is based on the following weight of evidence considerations:

- the toxicity observed during the appropriate duration of concern (short/intermediate term) were observed only at high doses [LOAELs = 900 mg/kg/day in the dog, 1000 mg/kg/day in the rat, and 1212 mg/kg/day in the reproduction study],
- the brain lesions observed in the chronic dog study are not appropriate for this scenario since the brain lesions were seen only at termination (i.e. after exposure for 1-year) and toxicity observed after chronic exposure is not appropriate for application to the short/intermediate term scenarios,

- the brain lesions observed in the chronic study are not expected to occur after short term exposure due to the lack of similar lesions or associated toxicity (i.e., clinical neurologic signs) in the 90-day studies in rats or dogs,
- the cause for concern for the brain lesions is low because of the minimal lesion severity, lack of associated changes (i.e., gliosis, myelin loss astrocyte hypertrophy, neuronal necrosis etc.), and the non-specific nature of the lesions (i.e., focal vacuoles),
- the overall toxicity profile of this chemical clearly shows that toxicity is observed only at high doses (lowest LOAEL = 360 mg/kg/day in the chronic dog study), and
- the *in utero* endpoint identified in the rabbit study is not appropriate for this population of concern (children) (D392798, M. Hawkins, 11/23/2011).

3.5 Dermal and Inhalation Short- and Intermediate-Term:
Study Selected: developmental rabbit study
MRID No.: 41869524
Summary: See Appendix A, Guideline 83-3
Uncertainty Factor(s): 100X [10 interspecies; 10X intraspecies] FQPA SF = 1X, UF_{DB} = 1X.
Comments about Study/Endpoint/Uncertainty Factor: The developmental rabbit study was selected for both the short- and intermediate-term dermal and inhalation exposure scenarios with a NOAEL of 60 mg/kg/day and a LOAEL of 360 mg/kg/day.
An appropriate dermal study, showing no adverse systemic effects up to the limit doses and an inhalation study were not available for these endpoints. However, the short- and intermediate-term dermal and inhalation endpoints selected for adults are not applicable to children because the adult endpoints are based on *in utero* effects. HED reviewed the full toxicological database for trinexapac-ethyl in an attempt to select endpoints for children for the dermal and inhalation exposure scenarios; however, no appropriate endpoints were identified for these scenarios based on the following reasons:

- dermal irritation effects are mild after 21/28 days of exposure in the rabbit study,
- dermal irritation effects after a single exposure disappear after seven days,
- there are no systemic effects up to the limit dose in the dermal rabbit study,
- there are no adverse toxicological effects (at doses close to the limit dose of 1000 mg/kg/day) in the 2-generation reproductive study in rats or in the chronic/carcinogenicity studies in rats and mice,
- there is no maternal toxicity in the developmental toxicity studies in both rats and rabbits, and
- there are no identifiable endpoints for the oral studies in the rat or rabbit.

The DAF of 77.5% (MRID 42238105) and an inhalation absorption rate of 100% were applied to the NOAEL for the assessment of the dermal and inhalation risks, respectively.

3.5 Dermal and Inhalation Short- and Intermediate-Term: Based on the use pattern, long-term exposures are not expected.

A.4 Executive Summaries

Subchronic Rat Study

In a subchronic oral toxicity study [MRID 41563921], 15 Sprague-Dawley [Crl:VAF/Plus CD® rats/sex/dose were administered CGA 163935 Technical [96.9%] *via* the diet at concentrations of 0 ppm, 50 ppm, 500 ppm, 5000 ppm, or 20,000 ppm for 13 consecutive weeks. The mean daily intakes were 3, 34, 346, or 1350 mg/kg/day for males and 4, 38, 395, or 1551 mg/kg/day for females, respectively.

There was no adverse effect of treatment on survival, and no apparent treatment-related clinical signs of toxicity. At 20,000 ppm, both sexes displayed slight decreases in body weight throughout most of the study [93%-94% of control at study termination], and overall body-weight gains were reduced in both sexes [males 93%/females 89% of control]. Both sexes at the 20000 ppm dose level displayed decreased food consumption throughout the study, with the largest deficit occurring initially suggesting a palatability problem.

There were no treatment-related ocular changes in either sex. There were no apparent, treatment-related effects on the hematology or clinical chemistry parameters monitored in either sex. The urinalysis assessment found decreased urinary pH values in both sexes at 20,000 ppm.

There were no treatment-related effects on organ weights in either sex. There was an increased incidence of renal tubular changes [basophilia (3, 2, 1, 7, 13***), hyaline droplets (5, 7, 7, 11*, 13**), casts (2, 2, 0, 2, 6), with increasing dose] in males at 5000 ppm and 20,000 ppm, which was said to represent a shortened time of onset of spontaneous senile nephropathy, a common condition in aging rats. However, based on the fact that the incidence of senile nephropathy was comparable among the male groups in the chronic rat study at dose levels up to 20,000 ppm for 104 weeks indicates that these findings should not be considered adverse.

The systemic toxicity NOAEL is 20,000 ppm [males 1350 mg/kg/day; females 1551 mg/kg/day], the highest dose tested.

This guideline subchronic oral toxicity study is classified **ACCEPTABLE**, and it satisfies the guideline [OPPTS 870.3100; §82-1] for a subchronic oral toxicity study in the rat.

COMMENT: The original DER identified a no observed effect level [NOEL], which was based on the renal tubular changes in the males at 5000 ppm [346 mg/kg/day] and 20000 ppm [1350 mg/kg/day].

Subchronic Dog Study

In a subchronic oral toxicity study [MRID 41563920], 4 beagle dogs/sex/dose were administered CGA 163935 Technical [96.9%] *via* the diet at concentrations of 0 ppm, 50 ppm, 1000 ppm, 15,000 ppm, or 30,000 ppm for 13 consecutive weeks. The mean daily intakes were 2.0, 34.9, 515.9, or 927.1 mg/kg/day for males and 1.9, 39.8, 582.4, or 890.8 mg/kg/day for females receiving 50, 1000, 15,000, or 30,000 ppm, respectively.

There was no adverse effect of treatment on survival, and no apparent treatment-related clinical signs of toxicity other than few feces and emaciation. Both sexes at the 30000 ppm dose level displayed decreased food consumption throughout the study and, consequently, body weights [males 74%/females 88% of control at termination] and body-weight gains [negative weight gain

compared to control (males –0.325 kg vs 1.775 kg (control)/females –0.075 kg vs 1.225 kg (control)] were decreased throughout the study also.

There were no treatment-related ocular changes in either sex. There were no apparent, treatment-related effects on the hematology, clinical chemistry, or urinalysis parameters monitored in either sex.

The treatment-related effects on most organ weights in both sexes at the 30,000 ppm dose level are considered to be changes that would be expected due to decreased body weight/body-weight gain. Both sexes at the 30,000 ppm dose level displayed decreased popliteal lymph node weights [absolute and relative-to-brain]. Non-significant decreases in thymic weight were reported in males at 30,000 ppm. No other treatment-related organ-weight effects were reported in either sex.

Diffuse thymic atrophy was observed in all dogs [both sexes] at the 30,000 ppm dose level compared with none of the controls [either sex]. Focal thymic atrophy was not observed at the 30,000 ppm dose level but was observed in 3 of the 4 male controls (2-3 males in all other groups) and all of the female controls (2-3 females in all other groups). It was noted that the diffuse thymic atrophy observed at 30,000 ppm most likely occurred secondary to the nutritional imbalances related to reduced food consumption and body-weight loss.

The systemic toxicity NOAEL is 15,000 ppm [males 515.9 mg/kg/day; females 582.4 mg/kg/day], based on clinical signs (few feces and emaciation), decreased body weight/body-weight gain, and decreased food consumption in both sexes (related to the lack of palatability) and diffuse thymic atrophy at the systemic LOAEL of 30,000 ppm [males 927.1 mg/kg/day; females 890.8 mg/kg/day].

This guideline subchronic oral toxicity study is classified ACCEPTABLE, and it satisfies the guideline [OPPTS 870.3150; §82-1] for a subchronic oral toxicity study in the dog.

NOTE: In the original DER, the subchronic oral toxicity study in the dog was classified Supplementary, pending the submission of the 7-week pilot study for justification of the dose levels selected.

In a 7-week pilot study [MRID 41869523], beagle dogs [3/sex/group] were administered CGA 163935 technical (96.6%) via the diet at levels of 0, 500, 5,000, 15,000, or 15,000-50,000 ppm. At the highest dietary level, dogs were fed 15,000 ppm for 3 days, 30000 ppm from day 4 to 28, and 50,000 ppm from day 29 to 49. During the first 4 weeks of the 7-week pilot study, males at the highest dose level gained 42% less body weight than the control, and females at this dose level gained 12% less than control. During the week 4 to week 5 interval, both sexes at the highest dose level displayed a negative body-weight gain [males -0.80 kg/females –0.76 kg] compared to the positive body-weight gains in the controls [males 0.3 kg/females 0.23 kg] and other dose groups [males 0.07 kg to 0.13 kg/females 0.03 kg to 0.13 kg]. At study termination, decreased body weight/negative body-weight gains were observed at the highest dose level in both sexes [males 82% of control/-0.54kg; females 74% of control/-0.43 kg]. Food consumption was decreased throughout the study in both sexes at the highest dose level [males 68% and 30% of control; females 89% and 34% of control during weeks 1-4 and 4-7, respectively]. No apparent adverse effects were observed in hematology, clinical chemistry, or urinalysis parameters monitored. Absolute and relative thymus weights were decreased in females at the

highest dose level [14.5 g *vs* 3.4 g] only. In the kidney, tubular dilatation and tubular degeneration/regeneration were observed in all dogs/both sexes at the highest dose level compared to none in the control or other dose groups. Diffuse thymic atrophy was observed in all dogs/both sexes at the highest dose level and in 2 of 3 females at 15000 ppm.
Due to the (1) lack of palatability of the test material, (2) refusal of the dogs to eat diets containing greater than 30000 ppm of the test material, and (3) severe weight losses at 50000 ppm during weeks 4-7, it was concluded that 50000 ppm was too excessive for use in the subchronic (13 week) study. The average daily compound intake was 22.2, 218.7, 685.8, 956.2, and 733.6 mg/kg/day for males and 23.1, 214.3, 679.9, 1373.3, and 964.7 mg/kg/day for females at the 500 ppm, 5000 ppm, 15,000 ppm, 30,000 ppm, and 50000 ppm dose levels, respectively.
The dose levels used in the subchronic study are considered adequate [30,000 ppm], based on the 7-week pilot study [MRID 41869523; HED Document No. 009711].
Comment: Microscopic evidence of minimal, focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in all dogs at 20,000 ppm and in one male and two female dogs at 10,000 ppm was observed in the one year dog study. Although similar microscopic lesions in the brain were not reported in this 90-day study in dogs, there was an inconsistency in the level of sectioning of the brain [MRID 42779401] in the subchronic study, which precludes a definitive determination of the occurrence of these lesions following subchronic exposure. However, the chronic study identifies a NOAEL for these lesions [1000 ppm [males 31.6/females 39.5 mg/kg/day], which were observed at the LOAEL of 10000 ppm [males 366/females 357 mg/kg/day].

21-Day Rabbit Dermal Toxicity Study
In a repeat dermal toxicity study [MRID 41563922], 5 New Zealand white rabbits/sex/dose were administered CGA 163935 Technical [96.6%] *via* dermal application at dose levels of 0 (untreated), 0 (vehicle: dehydrated alcohol), 10, 100, or 1000 mg/kg/day for at least 21 consecutive days.
There was no adverse effect of treatment on survival, and no apparent treatment-related clinical signs of toxicity. There were no treatment-related, adverse effects on body weight, body-weight gain, or food consumption in either sex.

There were no treatment-related effects on any of the hematology parameters monitored in either sex. There were no apparent, treatment-related, consistent, adverse effects on the clinical chemistry parameters monitored in either sex, although several statistically-significant trends were noted at 100 mg/kg/day and 1000 mg/kg/day. For example, decreased mean total bilirubin was observed in both sexes on day 21, but there was no dose response. Higher albumin/globulin ratios were observed at termination in both sexes compared to both control groups, but statistical significance was not attained in females and a dose response was not observed in males.
There were no treatment-related effects on organ weights in either sexes. Microscopic evaluation of the liver, kidney, and lungs did not reveal any treatment-related effects in either sex.
There was a dose-related increase in gross lesions [dark, red or tan] of the treated skin, including the vehicle group. Erythema was observed in females at the mid- (5 of 5) and high- (4 of 5) dose groups compared to none in either control of low-dose groups. No microscopic findings of the

skin were observed in any of the untreated controls (both sexes). Dermal microscopic observations included subacute lymphocytic infiltration in all high-dose females and 2 of 5 mid-dose females compared to none in either control group or low-dose group, and acanthosis was observed in all rabbits at each dose level (both sexes) and in the vehicle control group (both sexes) compared to none in the untreated control groups.

The systemic toxicity NOAEL is 1000 mg/kg/day, the highest dose tested.

The dermal toxicity NOAEL is 10 mg/kg/day, based on subacute lymphocytic infiltration in females at the LOAEL of 100 mg/kg/day.

This guideline repeat dermal toxicity study is classified ACCEPTABLE, and it satisfies the guideline [OPPTS 870.3200; §82-2] for a repeat dermal toxicity study in the rabbit.

COMMENT: In the original DER, the NOEL [10 mg/kg/day] was based on effects on various biochemistry parameters [decreases in total bilirubin, increase in mean albumin/globulin ratios, and increase in mean phosphorus] and on microscopic observations of the skin [hyperkerotosis and subacute lymphocytic infiltrates]. The original reviewer [1990] concluded that no adverse effects were noted in the clinical chemistry parameters. Since that time, NOAELs rather than NOELs are established. The registrant submitted a request [MRID 46809310] to establish a NOAEL for this study, based on arguments that the clinical chemistry findings do not constitute adverse effects due to dermal exposure to trinexapac-ethyl. The updated Executive Summary incorporates this change.

Developmental Rat Study

In a developmental oral toxicity study [MRID 41563923], 24 pregnant Tif: RAIf (SPF), hybrids of RII/1 x RII/2 rats/sex/dose were administered CGA 163935 Technical [96.6%] *via* gavage at dose levels of 0, 20, 200, or 1000 mg/kg/day during gestation days 6-15.

There was no adverse effect of treatment on survival, and no apparent treatment-related clinical signs of toxicity. Body weight was not adversely affected at any dose level. At the high-dose level, the dams displayed a significant decrease in body-weight gain during GD 0-6 (prior to dosing; 92% of control) and GD 0-21 (overall; 94% of control). Corrected body weight and body-weight gains were comparable among the groups. There was no treatment-related effect on food consumption.

Pregnancy rates were comparable among the groups (pregnant prior to dosing). There were no maternal deaths, no abortions, and no premature deliveries. There was a statistically-significant decrease (89% of control) in the mean number of corpora lutea at the high-dose level [17.0] compared to the control [19.1]; however, the value is stated to be within the historical control range. Also at the high-dose level, the mean numbers of implantations (95% of control) and live fetuses (94% of control) were slightly lower than the control. The number of early resorptions was comparable among the groups, and there were no late resorptions. There were no dead fetuses. Pre- and post-implantation losses were comparable among the groups. Fetal body weights and sex ratios were comparable among the groups.

There were no treatment-related malformations. One fetus at 1000 mg/kg/day displayed hypoplasia of the testicle (no historical control data reported). There was an increase in the incidence of asymmetrically shaped sternebra at 1000 mg/kg/day [8 fetuses in 7 litters; litter

incidence: 29.2%] compared to the concurrent [2 fetuses in 2 litters; litter incidence: 9.1%] and historical [48 fetuses/35 litters; litter incidence: 15.1%] control groups.
The maternal toxicity NOAEL is 1000 mg/kg/day, the highest dose tested. The developmental toxicity NOAEL is 200 mg/kg/day, based on an increased incidence of asymmetrically-shaped sternebrae at the LOAEL of 1000 mg/kg/day (limit dose).
This guideline developmental toxicity study is classified ACCEPTABLE, and it satisfies the guideline [OPPTS §83-3, but not 870.3700 because it only dosed from GD 6-15] for a developmental toxicity study in the rat.

Rabbit Developmental

In a developmental oral toxicity study [MRID 41869524], 16-17 pregnant New Zealand White rabbits/sex/dose were administered CGA 163935 Technical [96.6%] *via* gavage at dose levels of 0, 10, 60, or 360 mg/kg/day during gestation days 7-19.
On pregnant doe at the high-dose level was found dead (convulsion) on gestation day [GD] 13. A second high-dose doe was sacrificed on GD 24 following abortion on GD 22 and severe weight loss (hemorrhagic depressions in the stomach). There were no treatment-related abortions, but one doe in each of the control, mid-, and high-dose groups aborted. There were no apparent treatment-related clinical signs of toxicity in any of the surviving does.

Body weight/body-weight gain and food consumption were not adversely affected in the survivors at any dose level.

Pregnancy rates were comparable among the groups (pregnant prior to dosing). There were no premature deliveries. The mean number of corpora lutea was comparable among the groups. At the high-dose level, the mean number of implantations [7.6±2.4 vs 8.8±2.7; 86% of control] and mean number of live fetuses [5.7±2.7 vs 7.7±2.8; 74% of control] was reduced compared to the control. The mean number of live fetuses at the high-dose level was below that of the historical control [6.1]. Total resorptions were increased at 60 (29) and 390 (27) mg/kg/day compared to control (22). Similarly, the number of early resorptions (19 and 14 at 60 and 360 mg/kg/day, respectively) were increased compared to control (7), and there were no dead fetuses. Pre- [24.3%] and post- [24.8%] implantation losses were significantly increased at the high-dose level compared to the control [14.3% and 13.2%, respectively]. Fetal body weights and sex ratios were comparable among the groups.
There were no treatment-related malformations or variations [external, visceral, or skeletal].
The maternal toxicity NOAEL is 360 mg/kg/day, the highest dose tested. The developmental toxicity NOAEL is 60 mg/kg/day, based on a decrease in the mean number of fetus/litter and an increase in post-implantation loss, and an increase in early resorptions at the developmental toxicity LOAEL of 360 mg/kg/day.
This guideline developmental toxicity study is classified **ACCEPTABLE**, and it satisfies the guideline [OPPTS §83-3, but not 870.3700 because it only dosed dams from GD 7-19] for a developmental toxicity study in the rabbit.

Reproductive Toxicity Study

In a 2-generation reproduction study (MRID 43128604), CGA 163935 Technical [96.2% a. i.] was administered to 30 Sprague-Dawley rats/sex/dose in the diet at concentration levels of 0, 10, 1000, 10,000, or 20,000 ppm [equivalent to 0, 0.91, 9.01, 130.81, 450.72, or 911.77 mg/kg bw/day (males)/ 0, 1.08, 10.66, 154.08, 538.73, or 1073.42 mg/kg/day (females)] for 78 weeks. There were no adverse or compound-related effects on mortality or clinical signs for either the P or F1 generation. At 20,000 ppm, decreased body weight [P males 92%**/females 83%** of control (day 91); F1 males 82%**/females 86%** of control (day 84)], body-weight gain [P males 87%**/females 62%** of control (days 0-91); F1 males 83%**/females 81%* of control (days 0-84)], and food consumption [P males 86%**-96%*/females 81%**-87%** of control; F1 males 87%**/females 86%**-93%** of control] were observed during the pre-mating period in both generations. At 10,000 ppm, there were slight decreases in body weight [P males94%*/females 93%* of control (day 91); F1 males 93%*/females 96% of control], body-weight gain [P males 90%**/females 85% of control; F1 males 90%**/females 94% of control], and food consumption [P males 93%*-98%/females 95% of control; F1 males 90%**-93%/females 94%-100% of control] during the pre-mating period. Although sporadic, significant, decreases in body weight [P males 93%-95%*/females 94%-96% of control; F1 males 92%*-95%/females 94%*] and body-weight gain [P males 93%/females 89% of control; F1 males 87%**/females 88% of control] were noted at 1000 ppm mainly in the males (both generations), there was no dose-response. Additionally, during week 4 the P 1000 ppm rats were inadvertently fed the 20000 ppm diet, which may have contributed to these weight changes.

Throughout gestation, dams at the 20000 ppm displayed decreased body weights [P generation 83%**-86%** of control; F1 generation 89%** of control] and body-weight gain [P generation 93%; F1 generation 89% of control (days 0-20)]. Food consumption was decreased initially also [P generation 90%** of control (days 0-7), 91%-92% of control (days 7-20); F1 generation 87%** (days 0-7)/91%* (days 7-14)/94% (days 14-20) of control].

Throughout lactation, there was a decrease in body weight at 20,000 ppm, although the deficit diminished with time. The overall body-weight gain (days 0-21) at 20,000 ppm was positive [P dams 30.5 grams **; F1 dams 21.1 grams **] compared to negative gains in the control and lower dose groups.

There was no adverse effect on reproductive performance in either generation [comparable male and female mating index, fertility index, gestation index, gestation duration, # and % pregnant, # implantation sites, # viable litters, # and % stillbirths, pre- and post-implantation losses].

There were no treatment-related adverse effects on organ weights, and no treatment-related differences in macroscopic and microscopic pathology for either generation.

There were no adverse effects on the mean litter size on day 0, and the % of males was comparable among the groups for both generations. In each generation, pup survival (days 0-4) at 20000 ppm was lower [F1 90.1%/F2 93.6%] than the control [F1 96.9%/F2 96.9%] and other dose groups [F1 96.3%, 93.4% 98.5% with increasing dose/F2 96.9%, 96.5%, 96.9%, 96.9% with increasing dose], although statistical significance was not attained. F1 pup survival (lactation days 4-21) was lower at 20000 ppm [92.4%*] than the control [98.3%] and other dose groups [97.8%, 100%, 98.3%, 98.0% with increasing dose]. F2 pup survival (lactation days 4-21) was comparable among the groups. Pup body weights were decreased at 20000 ppm for both generations on day 0

[F1 males 94%/females 95% of control; F2 males 95%/females 93%* of control] and throughout lactation [F1 males 76%**-82%**/females 77%**-82%** of control; F2 males 76*-83%**/females 76**-93%* of control]. Body-weight gains were decreased in both sexes/both generations throughout the lactation period at 20,000 ppm, with the largest deficit being observed initially (days 0-4; F1 males 39%/females 41% of control; F2 males and females 59% of control). The magnitude of the body-weight gain deficits was 74%-89% (F1 males), 76%-87% (F1 females), 70%-81% (F2 males), and 71%-79% (F2 females). Clinical observations of the pups during lactation did not demonstrate treatment-related systemic toxicity.

The NOAEL for reproductive toxicity is 20,000 ppm [males 1212 mg/kg/day; females 1485 mg/kg/day (highest dose tested; HDT)].

The NOAEL for parental systemic toxicity is 10,000 ppm (males 593.5 mg/kg/day; females 751.1 mg/kg/day), based on reduced premating and gestation body weight/body-weight gain and food consumption at the parental systemic toxicity LOAEL of 20,000 (males1212 mg/kg/day; females 1485 mg/kg/day).

The offspring systemic toxicity NOAEL is 10,000 ppm (males 593.5 mg/kg/day; females 751.1 mg/kg/day), based on decreased pup survival and decreased pup body weight/body-weight gain during lactation at the offspring systemic toxicity LOAEL of 20,000 ppm [males 1212 mg/kg/day; females 1485 mg/kg/day).

This 2-generation reproduction study in rats is **acceptable** (guideline), and it satisfies the guideline requirement for a reproduction study [OPPTS 83-4; OECD 416] in rats. Developmental milestone data (age of vaginal opening and preputial separation; anogenital distance for F2 pups); assessment of implantations sites; estrous cycle length and periodicity (F1 weanlings); and sperm measures were not included in this study.

Chronic Dog Study

In an oral chronic toxicity study (MRID 42779402, CGA-163935 Technical [*(96.2/92.2% a.i.),* batch/lot #s FL 882373, 892178, 891417] was administered to 4 purebred beagle dogs/sex/dose *via* the diet at dose levels of 0, 40, 1000, 10,000, or 20,000 ppm (equivalent to 0, 1.56, 31.62, 365.72, or 726.65 (males)/1.37, 39.54, 357.13, or 783.83 mg/kg bw/day) for 52 weeks.

All dogs survived until study termination. At dose levels of 10,000 ppm and/or 20,000 ppm, emesis with food (earliest occurrence at week 7 in males), mucoid/bloody feces (earliest occurrence at week 6 in males), reduced RBC and hematocrit, elevated cholesterol and alkaline phosphatase were observed in both sexes. Slightly lower body weights were observed at study termination in males at 10,000 ppm [89% of control] and 20,000 ppm [90% of control] and in females at 20,000 ppm [89% of control]. Body-weight gains fluctuated throughout the study especially in males such that a clear dose-response was not evident. Decreased body-weight gains were observed initially at 20,000 ppm in both sexes [males negative gain; females 14% of control during first week; males 55%/females 60% of control during weeks 1-13 interval]. Overall, males at 10,000 ppm [66% of control] and 20,000 ppm [76% of control] displayed the lowest body-weight gain, although there was no dose-response. Females at 20,000 ppm displayed a decrease in body-weight gain overall [73% of control]. Food consumption was not affected. Although not statistically significant, decreased spleen weights were observed in both sexes at 20,000 ppm. Testes (absolute*, relative-to-body/brain) and uterus weights (absolute*,

relative-to-body*/brain) were significantly decreased at the three highest dose levels, but there was no dose-response and no histopathological lesions in either organ. There was microscopic evidence of minimal, focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in all dogs at 20,000 ppm and in one male and two female dogs at 10,000 ppm. Dose levels up to 20000 ppm were well tolerated by both sexes and are considered adequate.

The LOAEL is 10,000 ppm [males 365.72/females 357.13 mg/kg/day], based on elevated serum cholesterol values in females, mucoid feces in females and bloody feces in both sexes, and minimal, focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain in both sexes. The NOAEL is 1000 ppm [males 31.62/females 39.54 mg/kg/day].

This chronic oral toxicity study in the dog is **acceptable**, and it satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in the dog.

Combined Chronic Toxicity/Carcinogenicity Study in Rats

In a chronic toxicity/carcinogenicity study [MRID 42238104], 50 Sprague-Dawley Crl:VAF Plus CD(SD)BR rats/sex/dose were administered CGA 163935 Technical [96.9%/96.2%/92.2%] *via* the diet at concentrations of 0 ppm, 10 ppm, 100 ppm, 3000 ppm, 10,000 ppm, or 20,000 ppm for 104 weeks [carcinogenicity phase]. An additional group of 20 Sprague-Dawley Crl:VAF Plus CD(SD)BR rats/sex/dose were administered CGA 163935 *via* the diet at the same concentrations for 104 weeks [chronic toxicity phase], and an additional group of 10 Sprague-Dawley Crl:VAF Plus CD(SD)BR rats/sex/dose were administered CGA 163935 *via* the diet at the same concentrations for 52 weeks [interim sacrifice]. The time-weighted mean daily intakes were 0.38, 3.87, 115.6, 392.7 or 805.7 mg/kg/day for males and 0.49, 4.88, 147.4, 494.0, or 1054 mg/kg/day for females receiving 10, 100, 3000, 10,000, or 20,000 ppm, respectively. A recovery phase of 4 weeks followed the administration of the test material to 10 rats/sex at the 20000 ppm dose level [compared with an additional control group of 10 rats/sex].

There was no adverse effect of treatment on survival, and no apparent treatment-related clinical signs of toxicity. There was a reduction in body weight in both sexes [males 91%-94%; females 86%-92%] at the 20,000 ppm dose level throughout the study beginning at day 7. At 13 weeks, both sexes at 20,000 ppm displayed an 8% decrease in body weight. At study termination, males at 20,000 ppm displayed a 9% deficit in body weight compared to the control. Females at this dose level showed a greater gain in body weight during the last 24 weeks of the study [week 76-104 interval] such that terminal body weight was comparable [98% of control] to the control; however, at week 76, females at 20,000 ppm displayed a 14% deficit in body weight compared to the control. Decreased body-weight gains were observed in both sexes at the high dose during the weeks 0-12 interval [males 93*%/females 84%** of control]. Females also showed decreased body-weight gain at the 1-year time point [81%**of control]. Overall body-weight gain was 90% of control for the males and 95% of control for the females at 20,000 ppm. Food consumption values were lower than the control values for the first 6 months in males and for the first 15.5 months for females.

There were no treatment-related ocular changes in either sex. There were no apparent, treatment-related effects on the hematology or clinical chemistry parameters monitored in either sex. Urinary pH was decreased in both sexes at the 10,000 ppm and 20,000 ppm dose levels throughout the study, although no histopathological correlations accompanied these findings.

In males, decreased absolute and relative-to-brain heart weights were observed at 20,000 ppm at 52 weeks. There was a slight but significant increase in relative liver and lung weights in females at the 20,000 ppm dose level at 52 weeks. At termination [104 weeks], the increased relative organ weights were considered due to decreased body weight at 20,000 ppm. No treatment-related organ-weight effects were reported in either sex at 10,000 ppm.
In the liver, there was a statistically-significant increase in bile duct hyperplasia in males at 20,000 ppm at study termination [16, 11, 13, 13, 18, 35** with increasing dose]. Females at 20,000 ppm displayed an increased incidence of acanthosis in the glandular stomach at study termination [7, 6, 1, 1, 8, 13* with increasing dose]. Lymphangietasis was increased at the two highest dose levels in males at study termination [3, 4, 8, 4, 9*, 19* with increasing dose]. Females displayed an increased incidence of galactocele of the mammary gland [5, 5, 4, 7, 9, 13** with increasing dose] and stromal hyperplasia of the ovary was increased at the two highest dose levels [3, 1, 3, 1, 7*, 5* with increasing dose], but there was no dose response.
Squamous cell carcinoma of the non-glandular stomach was observed in two males (2.5%) at 20,000 ppm but in none of the control rats or other rats at any dose level in either sex. The normal range reported for males by the author was 0%-1.2%. Follicular adenocarcinoma of the thyroid was significantly increased in males (5%) at 20,000 ppm, and there was a significant positive trend [1, 0, 0, 1, 1, 4*]. The historical control range was 0%-5% (1.8% mean) compared to 1.2% in concurrent control. The incidence of combined follicular adenomas and adenocarcinomas of the thyroid did not show a dose-related increase [5, 2, 3, 6, 6, and 7 with increasing dose]. Urinary bladder papilloma incidence was significantly increased in females at 20,000 ppm [0, 0, 0, 0, 1, 2* with increasing dose), but the increase was slight and the finding is considered incidental.
The systemic toxicity NOAEL is 20,000 ppm [males 806 mg/kg/day; females 1054 mg/kg/day], the highest dose tested.
This guideline chronic toxicity/carcinogenicity study is classified **ACCEPTABLE**, and it satisfies the guideline [OPPTS 870.4300; §83-5] for a chronic toxicity/carcinogenicity study in the rat.
Non-neoplastic lesions: There was no apparent increase in the incidence of any non-neoplastic lesion in either sex.
Neoplastic lesions: Squamous cell carcinomas were observed in the non-glandular portion of the rat stomach in two males at the 20,000 ppm dose level.
Discussion of Tumor Data: It was concluded that extrapolation of effects on the non-glandular portion of the rat stomach to possible deleterious effects of trinexapac-ethyl on the pharynx and/or esophagus (non-glandular areas) of the human was not appropriate. This was because trinexapac-ethyl would not be in contact with the human tissues for a significant period of time compared with how it would have been in contact with the rat stomach.
Adequacy of the Dose Levels Tested: The highest dose [20,000 ppm] is approaching the limit dose in males (806 mg/kg/day) and is greater than the limit dose in females (1054 mg/kg/day). The dose levels are considered adequate for the assessment of carcinogenicity potential in the rat.

Carcinogenicity Study in Mice

In a carcinogenicity study (MRID 43128603), CGA 163935 Technical [FLs 872026, 881224, 882373; 96.9%/96.2% a. i.] was administered to 70 CD-1 mice (Crl:CD-1(ICR)BR)/sex/dose in the diet at dose levels of 0, 7, 70, 1000, 3500, or 7000 ppm [equivalent to 0, 0.91, 9.01, 130.81, 450.72, or 911.77 mg/kg bw/day (males)/ 0, 1.08, 10.66, 154.08, 538.73, or 1073.42 mg/kg/day (females)] for 78 weeks.
There were no adverse or compound-related effects on mortality, clinical signs, body weight, food consumption, hematology, organ weights, or gross and histologic (including tumors) pathology. The decreased body-weight gain observed in the 7000 ppm female group initially was attributed to palatability. **A LOAEL was not attained. The NOAEL is 911/1073 mg/kg/day (highest dose tested; HDT).**
There was no treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate based on a lack of adverse effect at dose levels up to and exceeding the limit dose.
This carcinogenicity study in mice is **acceptable** (guideline), and it satisfies the guideline requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in mice.
Non-neoplastic lesions: There was no apparent increase in the incidence of any non-neoplastic lesion in either sex.
Neoplastic lesions: There was no treatment-related increase in tumor incidence when compared to controls.
Discussion of Tumor Data: There was no increase in tumors of any type in either sex of mouse.
Adequacy of the Dose Levels Tested: . Dosing was considered adequate based on a lack of adverse effect at dose levels up to and exceeding the limit dose.

Acute Neurotoxicity Screening Battery
In an acute neurotoxicity study (MRID 48764506), groups of 10/sex Crl:CD(SD) rats (6 weeks of age) were given a single gavage dose (10 mL/kg) oftrinexapac-ethyl (95.8% a.i) suspended in the vehicle (0.5% carboxymethylcellulose in water containing 0.1% Tween® 80), at dose levels of 0 (vehicle only), 500, 1000, or 2000 mg/kg, respectively and observed for 15 days. The animals were observed twice daily for mortality and moribundity. Individual body weights were recorded on study days -7, 0, 1, 2, 7, 8, 14, and 15; individual food consumption was recorded during study days 0-1, 1-2, and 7-8. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 10 animals/sex/group pretest and on days 0 (4 hours post dosing time of peak effect), 7, and 14. At study termination, 5 animals/sex/group were euthanized and perfused in situ for neuropathological examination. Tissues from control and 2000 mg/kg/day animals were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Brain weights and brain dimensions (excluding olfactory bulbs) were recorded. Animals not selected for in situ perfusion were euthanized and discarded without macroscopic examination.

All animals survived to study termination. There were no treatment-related clinical signs of toxicity. There were statistically significant lower mean body weight gains for the 2000 mg/kg

males and females over the first day following dosing (study days 0-1). This effect did not continue for the rest of the study. There were no effects noted FOB evaluations performed at the time of peak effect (approximately 4 hours post-dose) and on study days 7 and 14. The FOB evaluations collectively involved home cage, handling, open field, sensorimotor, neuromuscular, and physiological parameters. There were also no treatment-related effects on mean total and ambulatory locomotor activity counts for males and females at any dose level. No remarkable shifts in the pattern of habituation occurred in any of the test substance-treated groups when the animals were evaluated on study days 0, 7, and 14. No treatment related effects were apparent in brain weights or brain dimensions for the perfused animals and there were no treatment related neuropathological lesions.

Based on the data provided in this study, the NOAEL for Systemic Toxicity is 2000 mg/kg (limit dose) and the LOAEL was not established.

Based on the data provided in this study, the NOAEL for Neurotoxicity is 2000 mg/kg (limit dose) and the LOAEL for Neurotoxicity was not established, based on the absence of any treatment-related functional observational findings, locomotor activity changes, changes in brain weights or brain dimensions, and the absence of any neuropathological lesions.

This study is classified as **Acceptable-Guideline** and as such satisfies the guideline recommendations for an acute neurotoxicity study in rats (870.6200a; OECD 424).

Comment: This is an updated Executive Summary. The original Executive Summary was revised to change the NOAEL for systemic toxicity from 1000 mg/kg to 2000 mg/kg and the LOAEL from 2000 to not established because the only effect seen was decreased body weight gain in the absence of a corroborating effects on body weight; this is not sufficient to be considered evidence of an adverse toxic effect.

Subchronic Neurotoxicity Screening Battery

In a subchronic neurotoxicity study (MRID 48764507) Trinexapac-ethyl (96.6%/95.8%,SM05D180/ SM08E551) was administered to 12 Crl:CD(SD) rats (Charles River Laboratories, Inc., Raleigh, NC.)/sex/group at dose levels of 0, 3750, 7500, or 15000 ppm (equivalent to 0,233, 463, and 948 mg/kg bw/day for males and 0, 294, 588, and 1171 mg/kg/day for females, respectively) for 13 weeks. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded weekly. The functional observational battery (FOB) and locomotor activity parameters were recorded for all animals during pretest and then during study weeks 3, 7, and 12. FOB parameters included home cage, handling, open field, sensory, neuromuscular, and physiological parameters. Ophthalmic examinations were performed prior to the start of test diet administration and during study week 11. At study week 13, 5 rats/sex/group were

deeply anesthetized and were perfused in situ. Brain weights and brain dimensions (excluding olfactory bulbs) were recorded. Neuropathological evaluation of selected tissues from the central and peripheral nervous systems was performed on 5 animals/sex in the control and 15,000 ppm groups.

All animals survived to study termination. There were no treatment-related effects on clinical findings. There were lower mean body weight gains with corresponding reduced mean food consumption for the 15,000 ppm females during study days 0-14. Following study day 14 the mean body weight gains and food consumption for the 15,000 ppm females were similar to control for the remainder of the study. These effects were not considered adverse since they were small and short lived. The mean body weights, body weight gains, and food consumption for the 3750 and 7500 ppm males and females and the 15,000 ppm males were unaffected by treatment. Also, there were no effects noted on FOB evaluations or for mean motor activity (ambulatory and total) counts at study weeks 1, 3, 7, and 12. There were no treatment-related changes in brain weight or brain measurements, or macroscopic or microscopic observations.

Based on the data provided in this study, the NOAEL for Systemic Toxicity is ≥ 15000 ppm (948 mg/kg/day for males and 1171 mg/kg/day for females, respectively) which is the highest dose tested. The LOAEL is > 15000 ppm for both systemic toxicity and neurotoxicity based on the absence of any treatment- related functional observational findings, locomotor activity changes, changes in brain weights or brain dimensions, and the absence of any neuropathological lesions.

The study is classified as **Acceptable-Guideline** and as such satisfies the guideline recommendations for a subchronic neurotoxicity study in rats (870.6200b; OECD 424).

Metabolism and pharmacokinetics study (rat)

In a metabolism and pharmacokinetics study (MRID 41563927), the metabolism of [cyclohexyl-^{14}C] CGA-163935 was studied in male and female Crl: CD BR rats. The cyclohexyl-labeled compound was administered as a single intravenous (iv) dose of 0.91 mg/kg, as a single oral dose of 0.97 or 166 mg/kg or as a single oral dose of 0.97 mg/kg following a 14-day pretreatment with unlabeled CGA-169935 at 1 mg/kg/day. Recoveries of radioactivity in urine after oral dosing (94.5-97.3% of the dose) indicated extensive absorption of the compound from the GI tract. Total recovery of radioactivity 168 hours after treatment accounted for 97-99% of the dose in the orally doses animals, and up to 94.7-98.3% of the dose in the iv-treated rats. Among the orally dosed groups, approximately 94.5-97.3% of the dose was eliminated in urine and 1.0-2.4% of the dose was eliminated in feces. Most of the elimination occurred within the first 24 hours. No differences between the sexes or among dose groups were found. The low elimination of radioactivity in the feces by the iv-dosed group (1.1-1.6% of the dose) suggests that there is very little or no biliary excretion of the compound and/or its metabolites in rats. At sacrifice, total radioactive residues in the carcass was less than 0.28% of the dose in all groups. A preliminary

experiment indicated that by 72 hours after dosing, elimination of radioactivity in expired air amounted to less than 0.06% of the dose for the low-dose rats and less than 0.01% of the does for the high-dose rats.

Only one metabolite, CGA-179500, was found in the urine. Levels of CGA-179500 in urine accounted for 82.0-91.6% of the dose among the orally-dosed animals and for 74.1-75.3% of the dose among the iv-dosed animals. CGA-179500 is the carboxylic acid resulting from hydrolysis of the ester bond of parent CGA-163935.

The study is classified as Acceptable-Guideline and as such satisfies the guideline the guideline requirement for a metabolism and pharmacokinetic study [OPPTS 870.7485].

Dermal Absorption Study

A single dermal dose of 0.01, 0.1, and 1.0 mg/cm2 14C-CGA-163935 administered to male rats was rapidly absorbed, distributed and eliminated. The amount absorbed increased with duration of exposure. Using the direct procedure to calculate skin absorption, the average 14C-CGA-163935 absorbed within 24 hours was 64.9, 63.86, and 30.85% of the applied dose for the low-, mid-, and high-dose groups, respectively. Using the indirect procedure to calculate skin absorption, the average 14C-CGA-163935 absorbed within 24 hours was 56.83, 66.74, and 33.84% of the applied dose for the low-, mid-, and high- dose groups, respectively. The direct procedure is the more realistic procedure to use. A dermal absorption factor of 77.5% was estimated based on the sum of 56.5% absorbed after 10 hours in the low dose group (0.01 mg/cm^2), in addition to 21% of applied dose that was associated with the application site.

I**mmunotoxicity study**

In an immunotoxicity study (MRID 48444101), trinexapac- ethyl (96.6% a.i.; Lot no. SMO5D180) was administered to female B6C3F1 mice (10/dose) in the diet at dose levels of 0, 500, 2000, or 5000 ppm (0, 160.2, 613.7, or 1530.5 mg/kg/day, respectively) for 28 days. Animals were divided into two subsets, the splenic antibody-forming cell (AFC) group and the Natural Killer cell (NKC) group. In the AFC group, positive control mice were administered cyclophosphamide via intraperitoneal injection (50 mg/kg/day) once daily for 4 consecutive days (study days 24 through 27). On study day 24, all AFC group mice were immunized with 0.2 mL of sheep red blood cells (SRBC, $7.5x10^7$/mL) via intravenous injections. In the NKC group, positive control mice were administered anti-asialo GM1 via a single intravenous injection (0.2 mL/animal) on study day 27, approximately 24 hours prior to scheduled necropsy. On day 28, all animals were necropsied, gross pathology observations were performed and selected organs (spleen and thymus) weighted. The immunotoxicity assessment was evaluated with a splenic antibody-forming cell (AFC) assay and a Natural Killer cell (NKC) activity assay.

There were no treatment related effects on mortality, clinical observations, body weights, food

consumption, thymus and spleen weights, or macroscopic findings in either the AFC or NKC groups of animals.

The systemic toxicity NOAEL is 5000 ppm (1530.5 mg/kg/day, the highest dose tested), the LOAEL was not established.

In the AFC group, there were no effects attributed to trinexapac-ethyl on spleen cell numbers and anti-SRBC antibody-forming-cell (AFC) response in either specific activity (AFC/10^6 cells) or total activity (AFC/spleen) to the T-cell-dependent antigen SRBC. The positive control (cyclophosamide) produced statistically significant decreases in specific activity (-100%) and total spleen activity (-100%) in the female B6C3F1 mice as expected, when compared to the vehicle control animals.

In the NKC group, there were no statistically significant effects attributed to trinexapac-ethyl at any E:T ratio as compared to the vehicle controls. The positive control (anti-asialo GM1) significantly decreased the functional response of the NK cells, reaching the level of statistical significance at the E:T ratios of 200:1 and 100:1, when compared to the vehicle control animals

The immunotoxicity NOAEL for anti-SRBC AFC response and NK cell activity is 5000 ppm (1530.5 mg/kg/day), the LOAEL was not established.

This immunotoxicity study is classified **Acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800).